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BULLETIN OF THE TORREY BOTANICAL CLUB
VOL. 82, NO. 3, PP. 155–162 MAY, 1955

MOSS COVER AND RAINFALL INTERCEPTION IN FREQUENTLY BURNED SITES IN THE NEW JERSEY PINE BARRENS

EDWIN T. MOUL AND MURRAY F. BUELL

The New Jersey pine barrens region is pine-dominated as the result of the frequent occurrence of fires. The fires sweeping through destroy the above ground parts of much of the vegetation. Pines are favored, first because they are less susceptible to damage by fire than are the broad-leaf trees, and second because the seed bed largely of mineral soil after the burning of the litter is more favorable to the establishment of pine seedlings than of seedlings of the native broad-leaf trees. Those shrubs which are able to sprout readily from the base also persist in spite of fire. As a result of wildfires in the past much of the pine region consists of a forest of widely-spaced, fire-damaged pines in a matrix of very dense shrubbery.

Where fire has been prevented for a sufficiently long time, a community dominated primarily by oaks has taken the place of the pine forest. The oak community shows every evidence of perpetuating itself, and as such may be considered the climax of the region (Stevens 1940, Little and Moore 1949, Buell and Cantlon 1950).

From an economic point of view the occasional destructive fires that produce the characteristic "barrens" type or scrubby vegetation are undesirable. Also undesirable would be complete prevention of fire which would allow the progression of the succession from pine to oak. The oaks of the pine barrens region are much less valuable than the pine.

Consequently foresters have been anxious to find practical means not only to perpetuate but to improve the pine forests. The most promising silvicultural practice now appears to be the use of what has become known as prescribed burning (Little, Allen and Moore 1948). Light winter burning results in slow fires that creep through the woods consuming accumulated litter and at the same time killing back sprouts of shrubs as well as reproduction of hardwood trees. The ground becomes very open and, if the overstory is dense, even the herb layer remains sparse. There it is the moss layer alone, including mosses, lichens and liverworts, that actually increases in cover as a result of the burning (Buell and Cantlon 1953).

The objectives of the present study were threefold—(1) to learn which species of bryophytes and lichens are present and make the principal contributions to the moss layer, (2) to discover what growth-forms of terrestrial bryophytes are of primary importance and (3) to learn something

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about the role of this layer of the community in respect to its influence on the amount of water that gets into the ground.

Growth-forms of bryophytes have been classified by Gimingham and Robertson (1950). These authors have studied the relative predominance of their growth-forms in various habitats in England. Using their growth-forms, Hamilton (1953) has recently found evidence that such growth-form spectra based on cover measurements may be useful as indicators.

Little attention seems to have been paid to bryophytes and lichens as interceptors of precipitation. Porter and Woollett (1929) studied water retention of lichens, and their results are expressed as percent of water held in terms of dry weight of the lichen mats. They found that mats of *Cladonia rangiferina* may absorb as much as 4.5 times their weight in water before allowing moisture to pass to the soil beneath. On the other hand, Jack (1935) expressed moisture retention in terms of inches of rainfall intercepted. He used mats of *Cladonia* set on screens in standard 8 inch rain gauges and found that from 0.10 inches to 0.15 inches of natural precipitation will be held by the mat. Thus Jack's data show that the mat prevented all moisture from passing through it in one third of the natural rains that occurred during the period June 25 to August 15, 1934. The work was done during a normal rainy summer in the northern part of the lower peninsula of Michigan. It is perhaps worth noting in connection with the present study that Jack found that artificial rains resulted in retention of a greater amount of moisture, up to 0.20 inches, than under natural rain.

The field work of the present study was done in June 1952 on two of the 5-acre experimental plots which were established in 1937 for the study of prescribed burning by the New Jersey Department of Conservation and Economic Development and the United States Forest Service at Mt. Misery in the Lebanon State Forest, New Jersey. The two plots used were those designated 1A and 2B. These were among the same plots used by Buell and Cantlon (1953) in their study of the effects of prescribed burning. One of the plots selected had been burned annually since 1937, and the other had been burned once every three years. The oaks had not been cut on the 2.5 acre areas where the work was done.

The authors are indebted to Mr. H. C. Barksdale and Dr. S. Little for their critical reading of the manuscript and for their helpful suggestions.

Methods. Along straight lines established at intervals through the stand 250 2×5 dm. quadrats were established at two-meter intervals. Cover in the moss layer was estimated in each quadrat. This included cover contributed by not only bryophytes and lichens, but that consisting of litter and logs. In some instances recently fallen litter covered bryophytes, and in others bryophytes and lichens occurred on logs, so that total cover as recorded often totaled over 100% due to overlap. Absence of cover was

recorded as "space" so that a positive figure was obtained for the area of exposed ground surface.

Determination of the maximum amount of moisture that may be held by the moss layer was made by cutting uniform samples from lichen and moss mats. The samples were cut by using 1 lb. coffee cans as "cookie cutters." In the laboratory the samples were removed from the can intact and their area and depth carefully measured by averaging 5 diameters and thicknesses. The samples were placed on filter paper and then on wire screen which had been wetted and weighed. The whole was then sprinkled, allowed to set a while and then sprinkled again until thoroughly soaked. After dripping stopped, the sample with filter paper and wire was weighed. All samples were placed on a laboratory table and weighed every day for fifteen days. At the time of weighing the atmospheric temperature and humidity were recorded.

Results. Vegetation. Seven species of mosses, one liverwort and three lichens were recognized as being present on the quadrats. Three of these, *Dicranum scoparium, Ceratodon purpureus,* and *Polytrichum juniperinum,* are of primary importance, contributing a total of over 98% of the cover in the moss layer. *Leucobryum glaucum* is also important but is definitely secondary to these three. The other three mosses (*Thelia, Plagiothecium* and *Buxbaumia*) and the liverwort (*Frullania*) are extremely minor in their contribution to terrestrial cover. The species of *Thelia, Plagiothecium* and *Frullania* present are more characteristic of tree bases or logs.

Of the five principal growth-form types proposed by Gimingham and Robertson (1950) only three, mat, turf and cushion are present. No dendroid types nor wefts were found. Omitting *Buxbaumia,* which does not readily fit into the classification, 43% of the other species are the mat type, 43% the turf type and 14% cushion. The spectrum thus obtained from the floristic list of species present is almost meaningless in terms of the vegetation or the community structure. In terms of cover contributed by the life forms, the turf type represents over 98%, cushions nearly 1.5%, leaving about 0.5% for the mat type.

The lichens were tabulated in the field as either *Cladonia rangiferina, Parmelia* sp. or "*Cladonia uncinalis* and others." This last category was used since *C. uncinalis* occurred frequently dispersed among a variety of lichens not readily identifiable to species. The total lichen cover of the two plots averaged 17.6%.

The two plots differed in the amount of cover in the moss layer. The plot burned annually (1A) was found to have a lower cover. Its moss cover was 9% and lichen cover 8%, making a total of 17%. Twenty-nine percent was recorded as bare soil, the rest being the recent accumulation of litter since the last fire 16 months before the study was made. In contrast, the plot

which had been burned at three-year intervals (2B), the last time on March 10, 1949, had a moss cover of 29% and lichen cover of 19%, making a total of 48% cover in this layer. Only 13% of bare soil was exposed, the rest being covered by recent litter accumulation.

Interception. In terms of total depth of water, the moss mats sampled held well over a centimeter. The lichens retained less than a centimeter. Of the mosses, the one cushion type, *Leucobryum*, was the most effective in holding moisture. The sample used was 2.7 cm. thick and held a depth of 1.76 cm. of water, or .64 cm. per 1 cm. of mat thickness. All other moss samples were of the turf type. Although they were all thicker than that of *Leucobryum*, none held as much water, so that in terms of depth of water per cm. of mat thickness they prove to be less efficient. However, *Ceratodon*, which contributes quite appreciably to the moss cover, comes very close to *Leucobryum*, the sample holding 1.76 cm. of water depth, or .58 cm. per 1 cm. of mat thickness. The lichens do not intercept as much water as the mosses, but the amount is still appreciable in terms of the average summer storm.

The rates at which the samples soaked up water and dried out were different. *Leucobryum* was the slowest in both instances. When the dry samples were sprinkled, the water at first rolled off or collected in little pools on the uneven surface. All other mosses and the lichens took up water readily.

On drying, all samples continued to lose moisture for a while and then reached a point where they began to fluctuate with changes in atmospheric humidity. *Leucobryum* was the slowest to reach the point of moisture equilibrium with the atmosphere, a period of 10 days being required. All other mosses and *Cladonia uncinalis* reached this point in 9 days, but *C. rangiferina* required only 4 days.

There was a pronounced difference between the samples in their responses to atmospheric moisture. All weight changes were directly related to the changes in vapor pressure deficit of the atmosphere. *Ceratodon* and *Polytrichum* responded most with a maximum fluctuation of 12 grams and *Leucobryum* least with a fluctuation of 3 grams. The lichens were about equal with a fluctuation of 4 and 5 grams each, and only slightly more than *Leucobryum*.

Discussion. The pine region of the coastal plain of New Jersey is an important source of future water supply. Up to 20% of the volume of the coarser sediments may be occupied by water. Hence has come the concept of the pine barrens of southern New Jersey as an area underlain by a great reservoir, the last untapped source of water in the state (Barksdale 1952). The surface of this reservoir is the water table. The sands above the water table act as a natural filter for the annual increment of water and also as

a roof surfaced largely with the characteristic pine barrens vegetation. Any factors influencing the recharge of this water supply should be understood.

It is well known that vegetation both intercepts and uses considerable amounts of moisture. The degree of such interception and use varies considerably with the type of vegetation. Any change in the vegetation is likely to be reflected in the amount of water that ultimately penetrates to the water table. The natural succession in the pine barrens as already mentioned is toward an oak forest climax. Oak forests, though not extensive, are established in many parts of the pine region. Their conversion to pine forest, as well as the conversion of the much more pervading scrubby type of vegetation with scattered trees to a pine forest, may both be expected to influence the amount of water reaching the water table. For example, the pine canopy in contrast to the oak canopy may possibly intercept quite different amounts of rainfall. Cantlon's figures show that the crown of the pines he studied intercepted 19% of the rainfall of the summer of 1950, while oak crowns intercepted only 13% (Cantlon 1951). However, these figures do not necessarily represent total canopy interception, since the openings in the canopy of the mature oak forest may possibly never be comparable to those of a pine stand (Buell & Cantlon 1950). Little data are as yet available on the amount of interception contributed by the shrub layer and none on the effect of the herb, litter and bryophyte layers. However, figures are available on the pronounced degree to which prescribed burning reduces the cover of the shrub layer as well as its effects on herbs (Buell & Cantlon 1953).

It has also been shown how much the prescribed burning practice may be expected to change the forest floor layer of litter, bryophytes and lichens (Buell & Cantlon 1953). Burning, of course, practically eliminates litter but results in a decided increase in the bryophytes and lichens.

The results of the present study not only show that in some places up to 48% or nearly half the ground may be covered by a carpet of mosses and lichens, but that an appreciable amount of rainfall may be intercepted by them.

Cover in the bryophyte layer is not uniform by any means. In places both the lichens and mosses form a thin, patchy layer. There is, however, a large proportion of the ground covered by these where the mat is thick and continuous and of which the samples used in the moisture retention experiment are representative. The layer, while covering 48% of the ground on one plot burned every three years, covered only 17% on the one burned every year. Probably no significance should be placed on this difference between bryophyte cover of the plots used in this study, since it represents only one sample of each treatment and especially since notes made by foresters when the plots were established indicate that even then bryophytes were more important on plot 2B, the plot burned every third year. On the

basis of records that they made, the proportional increase in the bryophytes and lichens was actually greater on plot 1A. The average of the two plots, 33%, probably indicates the degree of development of this layer that may be expected in general as a result of the practice of winter burning.

The results of the determination of the maximum water retention by the mosses and lichens represent only a maximum that may be expected under ideal conditions where the mats dry out completely between wettings. Even under laboratory conditions the mats never attained a constant weight but fluctuated with changes in atmospheric humidity. Under field conditions this factor would undoubtedly play a larger part. Also the rapidity and degree to which mats growing on sand would lose moisture probably would not be the same as in mat samples on the laboratory table since they would be lying on sand that might be wetted and they could dry out only from above. Hence between showers the bryophytes and lichens may seldom attain the same degree of desiccation as the experimental samples. Also as Jack (1935) has shown, artificial wetting and natural rains do not necessarily have the same effect.

However, the laboratory study does give a suggestion of the probable role of the bryophyte-lichen mat in interception. The following is suggestive of the order of magnitude that may be expected. If we assume complete desiccation of the layer between storms and take the average figure for water absorption by the moss and lichen mats, we find that they may appreciably reduce the frequency with which some of the precipitation that penetrates the tree canopy may also enter the soil. Cantlon's figures based on standard type rain gauges for the summer and fall of 1950, from June 19 to November 14, show 28 days during which rain penetrated the forest canopy. Using the average figure of 0.62 inches of water retained by well developed moss mats and the average figure of 0.22 inches by lichen mats, we can get some idea of the importance of these two groups of plants. Such moss mats where present under pine could have prevented all water from reaching the mineral soil below them during all but 9 of the showers, if they dried out between showers. Under oak they would have prevented water from reaching soil in all but 8 showers. The lichen mats in the same way could have absorbed all throughfall during all but 11 of the showers under the pine and 14 under the oak. During this period the moss mats could have intercepted as much as 50% of the total throughfall where they occurred under pine, the lichens only about half as much. The figures are similar under oak. It should be emphasized, however, that where no bryophyte cover occurred, which averaged 67% of the ground area, all throughfall would percolate directly into the sandy soil.

The growth-forms of the bryophytes are significant for two reasons. First, as they relate to their effectiveness in interception and second as they

compare with growth-forms of terrestrial bryophytes on similar substrata but in different parts of the world.

Over 98% of the moss cover is represented by a growth-form classified as the turf type. Of the other 2% of cover more than 1% is by the cushion type and 0.5% by the mat type. It seems probable that the densely-packed upright plants of the turf and cushion types may be the most efficient growth-forms in the interception of moisture.

The development of a bryophyte layer with the turf type predominating on sandy substratum is especially interesting here, since in England the sandy substratum results in a similar growth-form spectrum. There Gimingham and Robertson (1950) found small turfs predominating on such soils.

In conclusion, it becomes obvious that prescribed burning has a very pronounced effect on increasing cover in the bryophyte-lichen layer. This layer makes a significant contribution to the total rainfall interception by the vegetation. But it is not yet known how interception by this layer compares with interception by the litter which it replaces. It is highly probable, however, that the total influence of the litter layer of areas not subject to prescribed burning would be much greater. The litter of such areas is not only a thick layer but forms a practically continuous cover in contrast to the average moss cover of 33% indicated by this study. It is also of interest that in this climate, with a high fire frequence and sandy substratum, a preponderance of the turf and cushion types of growth-form are favored. Although not yet shown experimentally, these, because of their closely-packed, upright leafy stems, may be the most effective types of bryophytes in rainfall interception.

SUMMARY

1. Prescribed burning, while removing the litter layer, favors development of a bryophyte-lichen layer. This layer may cover as much as 48% of the ground.

2. Three species of turf type represent 98% of the moss cover, one species of cushion type little over 1%, and three species of mat type cover about 0.5%. Lichens cover nearly as much area as bryophytes.

3. Under laboratory conditions, well developed moss mats of the kind studied when air dried may absorb an average of .62 inches of rainfall, ranging from .52 inches (*Dicranum*) to .68 (*Ceratodon* and *Leucobryum*). Well developed lichen mats may absorb from .15 inches to .28 inches depending on the species.

4. Assuming the mosses dry out completely between storms, they may intercept as much as 50% of the throughfall where they occur. Lichens may intercept about half as much as the mosses.

5. As an interceptor of precipitation the bryophyte layer, even at its

maximum, is, on an area basis, probably never as important as the thick, continuous litter layer of areas not subjected to prescribed burning.

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A SYSTEM FOR THE SYMBOLIC REPRESENTATION OF KARYOTYPES

EMILIO BATTAGLIA[1]

Many references to karyotypes in the literature indicate only the zygotic or gametic number of chromosomes. Descriptions of chromosome morphology, illustrated or verbal, are infrequent. There is an evident need for a method of expressing chromosome size and morphology by letters and numbers. The following system is therefore presented:

I) The adoption of a standardized system of symbols.

K = karyotype.

C = centromere.

S = satellite. A distinction might be introduced indicating by *S* satellites of large size, and by *s* small satellites.

Nt = nucleolar-thread. A slender thread, showing nucleolar condensation at telophase, that connects the satellite to the chromosome.

Nc = nucleolar constriction. Any constriction showing nucleolar condensation at telophase, but not thread-like ("nucleolar zone," not "nucleolar thread").

Anc = anucleolar constriction. Any constriction of the chromosome not involved in nucleolar condensation. If an anucleolar constriction has a thread-like form, the symbol *Ant* may be used.

The symbols mentioned above are of wide applicability. In some particular cases, however, new abbreviations can be introduced, thus for instance *ds* could be used to indicate a "differential segment" produced by cold treatment (in *Trillium*) and so on.

II) A standardized method for the diagrammatic representation of chromosome morphology.

a) Represent the centromere (*C*) as a truncate break in the chromosome (fig. 1).

b) Represent the nucleolar thread (*Nt*) as a line (fig. 1).

c) Represent satellites as spheres, a small sphere for a small satellite (*s*) (fig. 3); and a large sphere for a large satellite (*S*) (fig. 1).

d) Represent a nucleolar constriction, sat-zone not sat-thread, (*Nc*), as a transverse region of the chromosome subdivided by parallel, vertical lines (fig. 1).

e) Represent an anucleolar constriction, zone not thread, (*Anc*), as a transverse region of the chromosome (fig. 3).

[1] The author wishes to acknowledge his indebtedness to Dr. C. A. Berger for his criticism and assistance.

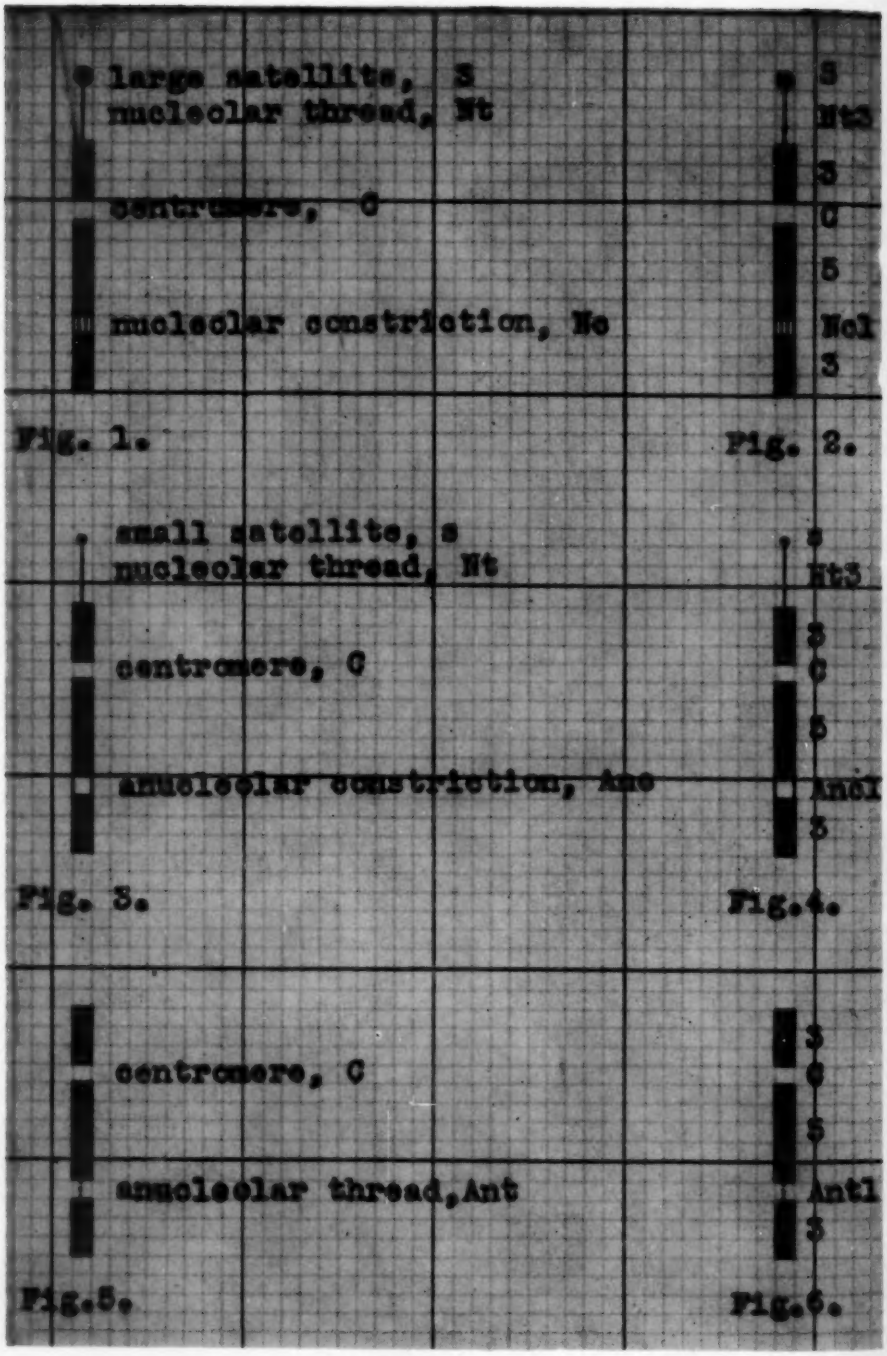


Fig. 1. Fig. 2.

Fig. 3. Fig. 4.

Fig. 5. Fig. 6.

f) Represent a thread-like anucleolar constriction, (*Ant*), as a dotted line (fig. 6).

g) Represent chromosome arms as segments with truncate ends (fig. 1, etc.).

Observe the following general rules:

h) Use graph paper ruled in 1mm squares,

i) Use a standard magnification of 2000×. If another magnification has been adopted write this magnification at the end of the formula,

j) Arrange the chromosomes in order of decreasing size from left to right, placing sex chromosomes, when present, at the left side, regardless of their size (the *X* chromosomes before the *Y*). The ends of long arms should be directed downwards and lying on the same abscissa. Starting from bottom each arm should be expressed by the number of the squares, or millimetres, representing its length; the centromere by the letter *C;* the nucleolar thread by the letters *Nt;* and so on. The length of the centromere, satellite, nucleolar constriction, nucleolar thread, etc., might be indicated by arabic numbers appended to the symbols. In such cases the symbols *C, Nc, Nt,* etc., and their lengths expressed in millimeters should be enclosed by commas. Thus a nucleolar constriction 3mm long will be represented by *,Nc3,*. To avoid too long formulae only the length of nucleolar threads and nucleolar constrictions should be mentioned, because of their relation to nucleolar size.

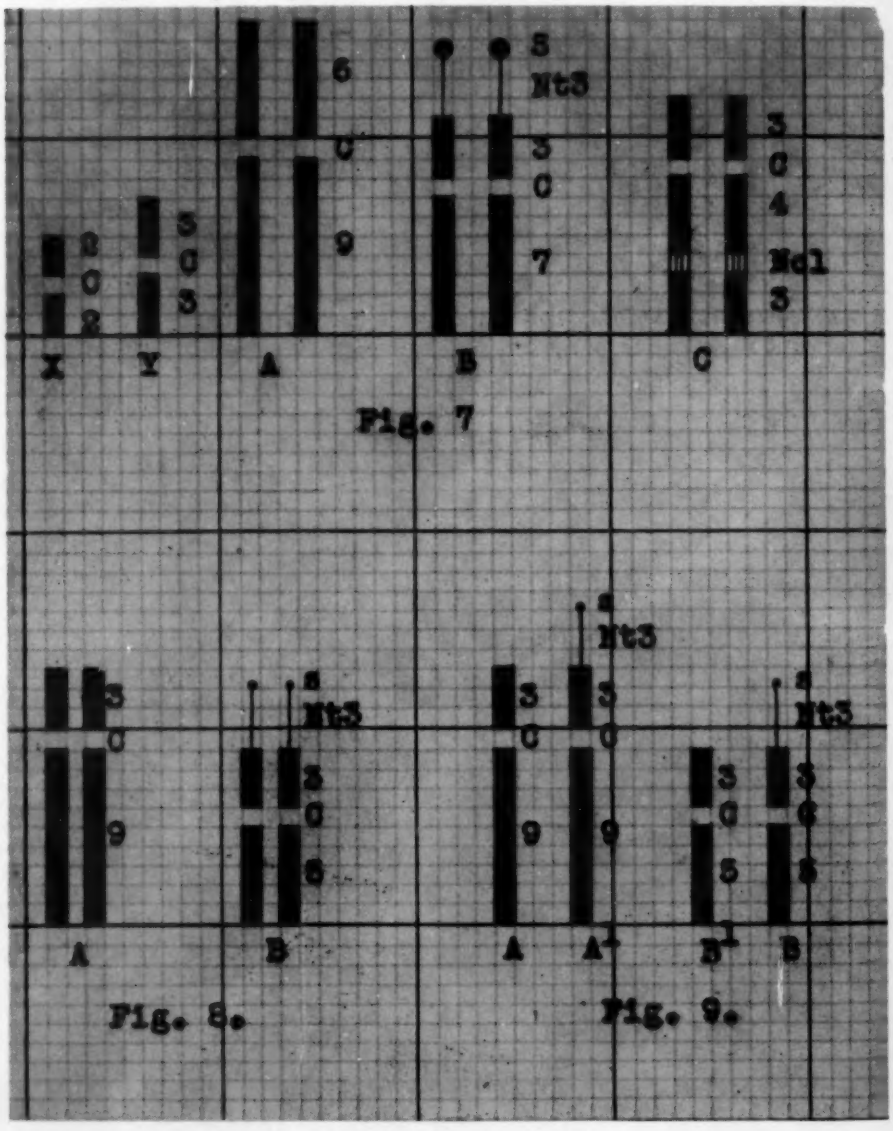
III) The expression of chromosome size and morphology in a sequence of letters and numbers (or formula).

Let us suppose a diploid organism with the zygotic chromosome complement 2n = 8 (fig. 7), namely a pair of chromosomes with submedian centromere (indicated by A), a pair of chromosomes with submedian centromere and a satellite on the short arm (indicated by B), another pair of chromosomes with submedian centromere and a nucleolar constriction on the long arm (indicated by C) and finally two short sex chromosomes, *X* and *Y* (indicated by *X* and *Y*). Figure 7 illustrates the correct diagrammatic representation of this karyotype. According to the proposed system the morphology of each chromosome would be adequately described by symbols as follows:

X = 2C2,
Y = 3C3,
A = 9C6,
B = 7C3,Nt3,S,
C = 3,Nc1,4C3.

The formula for this karyotype would be K = X(2C2) + Y(3C3) + 2(9C6) + 2(7C3),Nt3,S) + 2(3,Nc1,4C3).

Chromosome breadth (diameter at metaphase), being generally the same for all chromosomes of a karyotype, could be indicated at the end of the formula by adding the symbol d = 1 (case of figure 7 in which the breadth



of chromosomes is one millimeter, d = diameter). Supposing a magnification of 2000 ×, the completed formula will be:

K = X(2C2) + Y(3C3) + 2(9C6) + 2(7C3,Nt3,S) + 2(3,Nc1,4C3); d = 1; 2000 ×. Supposing the diameter of the X chromosome to be about one half

of a millimeter, the symbol of X in the above formula will be modified as follows: X(2C2),d = 1/2, that is: K = X(2C2),d = 1/2, + Y(3C3) etc.

Additional remarks.

a) Since chromosome length (at metaphase) is somewhat variable in different cells and tissues and after different methods of preparation, fractions of millimeters, in length, should not be used. However when fractions of a millimeter are significant, they may be added to the whole numbers, for example, ,Nt3½,.

b) In the case of heteromorphic chromosome pairs (fig. 8), represent each homologue as a distinct chromosome type and include both heteromorphic homologues between commas. Thus in an organism with two isomorphic chromosome pairs, (2n = 4), one pair of which has satellites, after translocation of a satellite we would have the following chromosome morphology (fig. 8) and formula:

Karyotype formula before the satellite translocation:

2n = 4 = 2A + 2B; K = 2(9C3) + 2(5C3,Nt3,s)

Karyotype after the satellite translocation:

2n = 4 = A + A^1 + B^1 + B; K = ,1(9C3) + 1(9C3,Nt3,s), + ,1(5C3) + 1(5C3 ,Nt3,s),.

By this system costly illustrations of karyotypes can be avoided since the idiogram can be reconstructed directly from the formula and vice versa.

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FOOT ROT OF TOMATO INCITED BY PHYTOPHTHORA CAPSICI[1]

P. D. CRITOPOULOS[2]

The disease was observed causing the death of mature tomato plants with half grown fruits, in large regions of a planting near Stockton, California. The affected plants had a rot at the base of the stem and at the tap root but most of the roots were not dead.

Three diseased tomato roots were sent for diagnosis to the Department of Plant Pathology of the University of California at Berkeley. The roots showed a dark discoloration and two out of the three yielded *Phytophthora* in culture. A pure culture was kindly given to the author for study by Dr. William C. Snyder.

According to reports in the literature the tomato plant can be parasitized, either naturally or after artificial inoculation, by the following *Phytophthora* species: *P. capsici* Leon. (Curzi 1927, Tucker 1931), *P. cryptogea* Pethyb. and Laff. (Pethybridge and Lafferty 1919, Bewley 1939), *P. drechsleri* Tucker (Tompkins and Tucker 1941a). *P. infestans* (Mont.) de Bary (Smith 1906), *P. mexicana* Hotson and Hartge (Hotson and Hartge 1923), *P. palmivora* Butler (Coleman 1910, Tucker 1931), *P. parasitica* Dast. (Sherbakoff 1917, Tucker 1931), *P. parasitica* var. *nicotianae* Tucker (Atanasoff and Kovacevski 1929, Tucker 1931) and *P. primulae* (Tomlinson 1952).

The type of disease produced is damping-off, root and foot rot, blight or buckeye rot. The buckeye rot can be caused by any one of six species, *P. capsici, P. cryptogea, P. drechsleri, P. infestans, P. mexicana* and *P. parasitica.* Natural infection of leaves is reported only for *P. infestans.* The plant part most commonly attacked is the stem at ground level.

The present investigation was undertaken to determine the symptoms of the tomato foot rot at Stockton, California, and to identify the pathogen, through the study of its morphology, pathogenicity and temperature-growth relation. The variability of the fungus and its survival in the soil were also investigated.

Materials and methods. The tomato plants were raised and inoculated in pots, kept in the greenhouse. These were grown singly in 20 cm. clay pots

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filled with ordinary soil. Both pots and soil were steam sterilized. The variety Bonny Best was used, unless otherwise specified.

The plants were inoculated at different stages of their development on the stem at soil level, either without wounding or through wounds, with mycelium of the pathogen growing in potato-dextrose agar. Non-inoculated controls were included in each test and the inoculant was re-isolated from all diseased specimens.

Detached immature and mature tomato fruits were inoculated with a mixture of zoospores and sporangia in water, which was prepared by straining the water from water cultures through 8 layers of cheese cloth. The fruits were inoculated with drops of the inoculum, placed directly on their unwounded surface, and were then placed in glass moist chambers. The inoculated fruits were removed from the moist chambers after different periods of incubation, and infection was allowed to develop either in the laboratory or out-of-doors. All infections were confirmed by culture methods.

Leaflets from the variety Ponderosa were tested for infection in Petri dish moist chambers. These were inoculated on one side of the midrib with a zoospore and sporangial suspension in water, which was poured off 5 hours later, and were then kept out-of-doors.

On leaflets from the same variety also kept in Petri dish moist chambers, the mode of penetration was studied. Inoculation was performed by spreading on their surface a thick suspension of zoospores and sporangia in water and the plants were then kept out-of-doors. Twenty-six and forty-eight hours after inoculation portions of inoculated areas from the leaflets were transferred for clearing to vials with saturated chloral hydrate solution. For microscopic examination, the material was mounted and stained in a 0.02 per cent solution of cotton blue in lactophenol.

The morphology of the pathogen was studied in water cultures. These were prepared by placing small squares, usually of pea-straw agar cultures, in Petri dishes containing a thin layer of sterile distilled water.

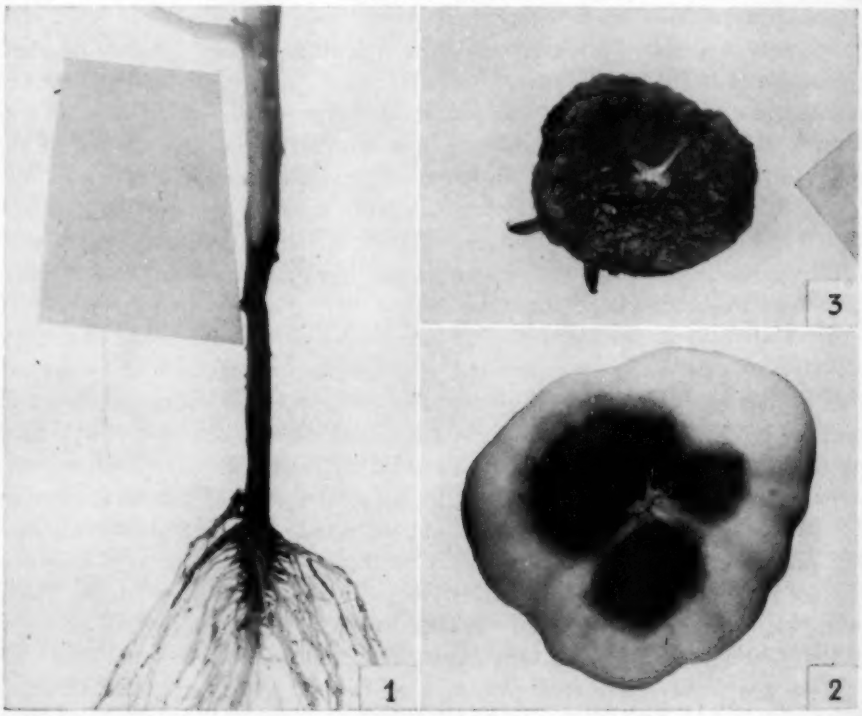
The temperature-growth relation was studied along with that of other *Phytophthora* isolates, in cultures growing in potato-dextrose agar plates. Each plate was prepared with 20 cc. of the medium. Disks of agar inoculum were cut with a small-sized cork borer from the edge of actively growing colonies in pea-straw agar plates. A disk was then transferred to the center of each plate and the plates were incubated at different controlled temperatures. The reading was taken 72 hours after the beginning of incubation. The growth of the mycelium was calculated by measuring its greatest and least distance from the inoculum. The average of these 2 readings was taken to represent the growth of the mycelium at a given temperature.

Single spore cultures of the fungus were obtained in tube slants with potato-dextrose agar, following the technique described by Hansen and Smith (1932).

The survival of the pathogen in the soil was tested by stuffing the soil into several varieties of healthy apples, potatoes or sweet pepper fruits, which were then kept out-of-doors. The invaded tissues were cultured in sterile distilled water, where the fungus could readily be identified.

Symptoms. When tomato seed was sown in soil infested with the isolate of *Phytophthora* from tomato foot rot, the majority of seedlings did not emerge above ground; a few that did, damped-off soon after.

Young plants inoculated without wounding, developed a dry foot rot and were thus killed in 5 or 6 days (fig. 1). Plants at the stage before bloom-



FIGS. 1–3. Symptoms on tomato produced by *Phytophthora capsici*. FIG. 1. The base of the stem and roots of a young plant of the variety Bonny Best, 20 days after inoculation at soil level without wounding, with mycelium growing in potato-dextrose agar. FIG. 2. Blossom end view of a 4 days old infection of an immature Ponderosa fruit inoculated with 3 drops of a zoospore suspension. FIG. 3. The same view of the same fruit after mummification, photographed 20 days after inoculation. Photographs by Victor Duran.

ing, when inoculated in the same way, developed a similar foot rot, which did not extend vertically more than 10 cm. The rotted portion of the stem was dark-brown and had no sharp demarcation from the adjacent healthy portion. The leaves exhibited permanent wilting without noticeable change in color. Such plants were killed in from 10 to 15 days.

In diseased plants, the rot often progressed through the pith from the soil level high up into the stem, while the pith below the soil surface remained healthy. Stems with decayed pith developed dry cankers at the axils of their leaves, as these slowly collapsed and died.

The main root of infected plants usually was partially decayed (fig. 1). From the finer root branches, some became decorticated, while others remained healthy. Several root branches were dead up to the point of their origin. On the surface of some roots arrested infection areas were found, in the form of brown spots or stripes.

The plants became more resistant as they matured. Mature plants escaped infection even when they were inoculated through wounds.

Small, immature tomato fruits infected with the pathogen developed at the point of inoculation firm, diffuse, brown spots, which were surrounded by a broad, water-soaked and light-brown border with ill-defined margin (fig. 2). The decay spread rapidly and the surface was covered with tufts of sporangia. The fruits shrank and became transformed into stone-like mummies in about 12 days (fig. 3). Full-sized, green fruits showed the same type of symptoms and signs, however, after reaching a certain stage of maturity; these were not mummified.

Mature fruits were not readily infected but once the infection started, it proceeded faster than it did on immature fruits. The infected fruits either did not have any browning at the point of inoculation or developed a brownish, diffuse discoloration. In the majority of lesions the border was water-soaked, slightly depressed and sharply demarcated from the healthy tissue. About 8 days after inoculation the surface of lesions was covered with compact tufts of sporangia and occasionally was marked with a yellowish pattern. Mature, infected fruits did not become mummified. Their rot was more or less soft.

When leaflets from young leaves were inoculated on one side of the midrib with a zoospore and sporangial suspension, the fungus invaded them entirely 2 days after inoculation, whereas in leaflets from older leaves the infection spread at a slower rate. The invaded areas were light-brown and portions of them were covered by evenly spread sporangia on short talks. In several tests where tomato plants were inoculated with the fungus, actual instances were recorded, where stem infections originated from leaves touching the infested soil.

The penetration of leaves by germ tubes from zoospores was determined to be predominantly direct, but it took place also through the stomata.

Experimental host range. In tests made to determine the host range of the pathogen, 3 crop plants from the host family were inoculated viz., potato (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.), and sweet pepper (*Capsicum frutescens* L.). Cowpeas (*Vigna sinensis* (L.) Endl.), Kid-

ney beans (*Phaseolus vulgaris* L.), peas (*Pisum sativum* L.), and Lima beans (*Phaseolus limensis* Macf.) were also tested for susceptibility to the fungus. The seed used for planting was surface disinfected with Spergon and the potatoes were washed in a solution of Chlorox.

In one of the trials, two potted plants of each of the seven species were inoculated when they were still young. The plants in one pot of each species were inoculated through wounds and those of the other were inoculated without wounding.

Through this trial it was determined that the fungus could readily kill both wounded and unwounded pepper plants. All wounded and 30 out of 43 unwounded plants were dead 66 hours after inoculation. The infection in these plants extended upward in the stem but spread very little or not at all in the root system. Twenty days after inoculation only 2 out of 43 unwounded pepper plants were still alive. The wounded Kidney bean plants shrank at their base without much discoloration and died in from 4 to 7 days, while the unwounded collapsed in from 5 to 8 days. A wounded pea plant was also killed by the fungus. Young plants of potato, tobacco, cowpea, and Lima bean, both wounded and unwounded, failed to become infected.

Thirty-seven days after the first trial, another one was conducted, in which 2 pots of each of the species used in the first trial and from the same seeded lot, were inoculated in the same way.

Eight days after inoculation the wounded and a few unwounded mature pepper plants were dead. Thirty-five days after inoculation all of the unwounded plants had died. From the mature pea plants two wounded and one unwounded collapsed through infection, 16 days after inoculation. Mature Kidney bean plants, both wounded and unwounded, had arrested infection lesions on their stems adjacent to the soil level but otherwise appeared healthy. Microscopic examination of sections through these lesions showed the presence of stout hyphae. The pathogen was identified in water cultures with tissue from the arrested lesions, made 30 days after inoculation. The isolate was non-pathogenic to mature plants of potato, tobacco, cowpea and Lima bean.

Morphological characteristics. The isolate of *Phytophthora* from tomato foot rot produced in agar media, pockets of profusely branching and interwoven hyphae, some of which had greater diameter and superficial tumescences. Hyphal swellings were produced in agar media as well as in water cultures. These were small, subsphaerical, isolated, intercalary, richer in protoplasm and germinated by germ tubes.

The production of sporangia was mostly studied in water cultures. Young sporangia were sphaerical but as they matured, a beak was formed at the side of the obtuse papilla, which was either apical or lateral. The

beak was long and straight; sometimes it was curved, and occasionally it formed a tube with walls vertical to the sphaerical sporangium. Up to 4 beaks were observed on a single sporangium. Mature sporangia were limoniform to irregular or elongate; some were sphaerical to ellipsoidal.

The sporangia varied in size from tiny ones, which approached the size of a single zoospore, up to the normal size, the mean of which was $55 \times 34\ \mu$. Occasional intercalary, beaked sporangia were produced. Sporangia formed on the surface of sweet pepper fruits were broadly ellipsoid and more uniform in shape, than those produced in water.

All sporangia seemed to have 1 or 2 pink sap vacuoles. When zoospore differentiation was well advanced, these vacuoles usually could not be observed, apparently because they were situated in the middle.

In water cultures kept out-of-doors during the summer when the temperature varied around 14° C, many sporangia with undifferentiated content were abstricted with their pedicel, which varied up to 170 μ in length.

Germination of sporangia. When the sporangia discharged in water cultures, the zoospores usually were pushed out rapidly one after the other. In passing out through the opening occasionally they were stretched considerably, assuming a length 4 times their width. The number of zoospores produced by a single sporangium varied according to its size. A medium sized sporangium was observed to liberate 38 zoospores, while a large one released 82. In the same cultures the content of other sporangia was extruded slowly, without being previously differentiated into zoospores and gradually became dispersed in the water.

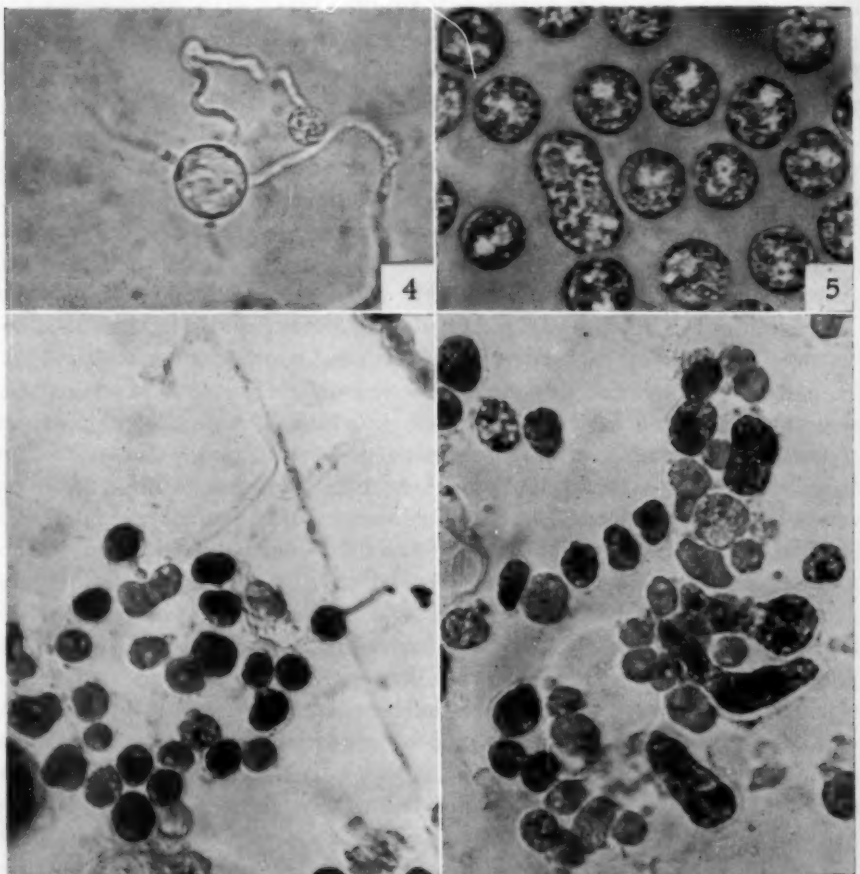
The zoospores were discharged by action apparently taking place within the sporangium. It seems probable that the sap vacuole is involved in this process. Once during the discharge of a sporangium the sap vacuole was observed to swell and contract instantaneously.

Direct germination of sporangia was frequently observed in solid media and occasionally in water cultures. Sporangia lying on the surface of cornmeal agar plate cultures produced through their wall several germ tubes of varying diameter. Assuming that the fusion of mature zoospores (Critopoulos, 1951) of a sporangium lying on the surface of an agar medium, can take place within the sporangium, the tubes with the greater diameter probably were produced by fusion bodies.

Zoospores and their germination. It is significant that the pathogen as a rule did not produce motile zoospores in water cultures with sterile distilled or tap water, at temperatures varying from 10°–25° C. Isolates from other species of *Phytophthora* tested under similar conditions, produced motile zoospores.

When a sporangium discharged in a water culture, the zoospores dropped slowly to the bottom of the plate or drifted for a while in the water.

At the bottom of the plate they exhibited an occasional, small, jerky movement more noticeable in some of them than in others. During summer their jerky movements were more vigorous. A few zoospores were observed to swim spirally, even 12 hours after they were cropped from the water cultures.



FIGS. 4–7. Zoospores and fusion bodies of *Phytophthora capsici* and their germination. FIG. 4. A fusion body germinating in water with 4 germ tubes; on its right, a germinating zoospore; enlarged. FIG. 5. Two zoospores fusing in water; much enlarged. FIGS. 6 and 7. Zoospores and fusion bodies on pea-straw agar. Fixed over osmic acid and stained overnight with 0.005% cotton blue in lactic acid. × 1360. Photographed by Prof. James T. Barrett.

When the zoospores were liberated they were variously lobed. The majority did not have any flagella and a few had only one. In all flagella observed there was a knob a short distance from the point of attachments. In from 5

to 15 min. the zoospores assumed a rounded shape, and stuck to the bottom of the plate. The rounded zoospores had a diameter of 9.2 to 10 μ.

In water cultures the majority of zoospores germinated at the bottom of the plate. The germ tubes lay on the surface of the plate or more frequently grew vertically in the water. These stuck to the bottom only through the zoospore wall. Several germ tubes were branched.

Pairing of zoospores. Right after discharge, the nonmotile zoospores at the bottom of a water culture plate, either approached each other by their jerky movements or withdrew. Two touching zoospores often united within 10 or 20 min. to form 1 larger, perfectly sphaerical body, with darker protoplasmic content (figs. 5 to 7). The size of this body remained unchanged or diminished right after pairing. When it was diminished, it always remained greater than the size of an unpaired zoospore. The average diameter of the fusion bodies was 13.8 μ. Occasionally their diameter increased and their protoplasmic content became thinly and evenly dispersed. The pairing of zoospores took place throughout the year. In summer, however, it proceeded at a slower rate.

Some of the single zoospores, as well as some of the paired, enlarged and then exploded. In one instance two touching zoospores were observed to complete their union in four minutes, assuming a perfectly sphaerical shape, and then exploding. In another case two zoospores united and before their union was complete, the two united with a third zoospore. Half an hour later the three united zoospores had the form of a budding yeast cell. In another instance a large cell, apparently derived from the fusion of two zoospores, was observed to unite with a zoospore of normal size. The resultant large body exploded a few minutes later.

During winter the paired zoospores, whose diameter was decreased, proceeded to germinate by a germ tube either immediately following fusion or twenty minutes after fusion; one of them was observed to produce 4 germ tubes (fig. 4). The unpaired zoospores germinated in the same way, usually after a longer period. The diameter of the germ tube of paired zoospores was larger than that of single zoospores.

In winter the paired zoospores could be identified easily one hour after discharge because many of them germinated at the bottom of the plate, whereas the unpaired and the paired ones, whose diameter increased, did not. Early in summer both unpaired and paired zoospores germinated, after assuming a round shape, by a germ tube at the same time. The pairing of zoospores and their germination or exploding varied considerably in winter as compared to summer. A number of cells derived from the pairing of two zoospores did not germinate even 24 hours after pairing.

Oogonia and antheridia. The isolate under study was found not to pro-

duce oospores in diseased plant tissues and did not produce sexual organs even after 2 or 3 months in any of the following artificial media: pea-straw agar, potato-dextrose agar, Leonian's agar, corn-meal agar and oat-meal agar.

By pairing it with a female strain of *P. drechsleri* secured from foot rot of Kidney bean at Brentwood, California, hybrid oospores were obtained. Thus the fungus was apparently a male strain. The antheridia were of the permanent, amphigynous type, encircling the stalk of the oogonium. The hybrid oospores had a diameter of from 25 to 35 μ, were brown and slightly wrinkled. Typical chlamydospores were not produced.

Effect of temperature on the mycelial growth of the isolate under study and other Phytophthora isolates. The growth rate of the pathogen

TABLE 1.—*Average radial growth in mm. of the mycelium of 6 Phytophthora isolates after 72 hours in potato-dextrose agar plate cultures under different temperatures (Centigrade).*

Pathogen	4°	10°	15°	20°	26°	31°	35°	41°	43°
Isolate from Tom. foot rot Stockton, Calif.	0	1.0	7.5	13.0	20.5	17.5	13.7	0	0
P. drechsleri Bean foot rot Brentwood, Calif.	0	2.7	13.0	17.5	24.0	25.5	22.5	0	0
P.drechsleri Tom. fr. rot Concord, Calif.	0	4.5	6.0	11.7	26.5	21.0	17.5	0	0
P. parasitica Tom. root rot Fayetteville, Ark.	0	0.5	11.5	16.0	22.0	30.0	16.5	0	0
P. parasitica Tom. fr. rot Brentwood, Calif.	0	1.5	9.0	14.0	21.2	23.0	14.5	0	0
P. erythroseptica Pink rot of pot. Stockton, Calif.	0	4.5	13.5	19.0	23.5	8.5	0	0	0

in potato-dextrose agar plate cultures at different temperatures was studied along with the following isolates. 1) *P. drechsleri* from foot rot of Kidney bean collected at Brentwood, California, 2) *P. drechsleri* from buckeye rot of a green tomato fruit at Concord, California, 3) *P. parasitica* from root rot of tomato at Fayetteville, Arkansas, 4) *P. parasitica* taken from buckeye rot of a green tomato fruit at Brentwood, California, and 5) *P. erythroseptica* from pink rot of a potato tuber at Stockton, California. The results obtained are presented in the table 1.

In this table a comparison can be made of the effect of different temperatures on the mycelial growth of the isolates studied. At 40° C. no growth resulted. After leaving the cultures at this temperature for the following

18 days, the isolate under study and the isolates of *P. drechsleri* and *P. parasitica* exhibited no growth, while *P. erythroseptica* showed a radial growth of 3 mm.

The isolates that did not show any growth after the 18-day period, were transferred to an incubator at 26° C. to test whether the inoculum had survived. After 15 days at this temperature it was determined that the isolates of *P. drechsleri* and *P. parasitica* had survived, while the isolate from tomato foot rot did not.

P. erythroseptica did not grow at 35° C. or above, while for the isolate studied and the isolates of *P. drechsleri* and *P. parasitica* no growth resulted at 41° C. or above. After the 72-hour incubation period at 41° and 43° C., the cultures of all 6 isolates were stored for 19 days at 14° C. Then these were incubated at 26° C. to test whether any one of the isolates was still alive. After 14 days at that temperature no one exhibited any growth.

The effect of temperature on the growth rate of the mycelium of the isolate from tomato foot rot can be summarized as follows: It grew at temperatures ranging from 10° to 35° C., with greatest amount of growth occurring at 26° C. It did not survive after remaining 72 hours at 4° or 41° C. A temperature-growth curve of this isolate along with the curves of the other isolates is given in figure 8. It should be noted that for most isolates, there was a sharp decrease in growth as the temperature increased above the optimum.

Identification of the pathogen. The *Phytophthora* isolate from foot rot of tomato at Stockton, California, was identified as *Phytophthora capsici* Leon. by the morphology of its mycelium, the papillate, limoniform to irregular or elongate sporangia with a mean size of $55 \times 34\ \mu$, the absence of typical chlamydospores and the production of amphigynous antheridia.

P. capsici, according to Tucker (1931), differs from all other species of the genus in attacking wounded well-grown stems of pepper plants. The pathogen studied invaded and killed young or mature stems of unwounded pepper plants, promptly after inoculation. The same author regarded *P. capsici* as having temperature relations very similar to *P. parasitica*. The temperature relations of the pathogen corresponded well with those of the latter.

P. drechsleri, two isolates of which were used for comparison in the study of the temperature-growth relation, has temperature relations similar to those of *P. parasitica* and *P. capsici*. It is readily distinguishable, however, from *P. parasitica* by the absence of chlamydospores and from both by its non-papillate sporangia, which usually develop sparingly even when the fungus is growing in water cultures.

Observations on variability. Sixty-six single zoospore cultures of the fungus were made in tube slants and the cultural characteristics of the

mycelia produced were compared under laboratory temperatures of approximately 22° C. The only character found useful for comparison was the rate of mycelial growth.

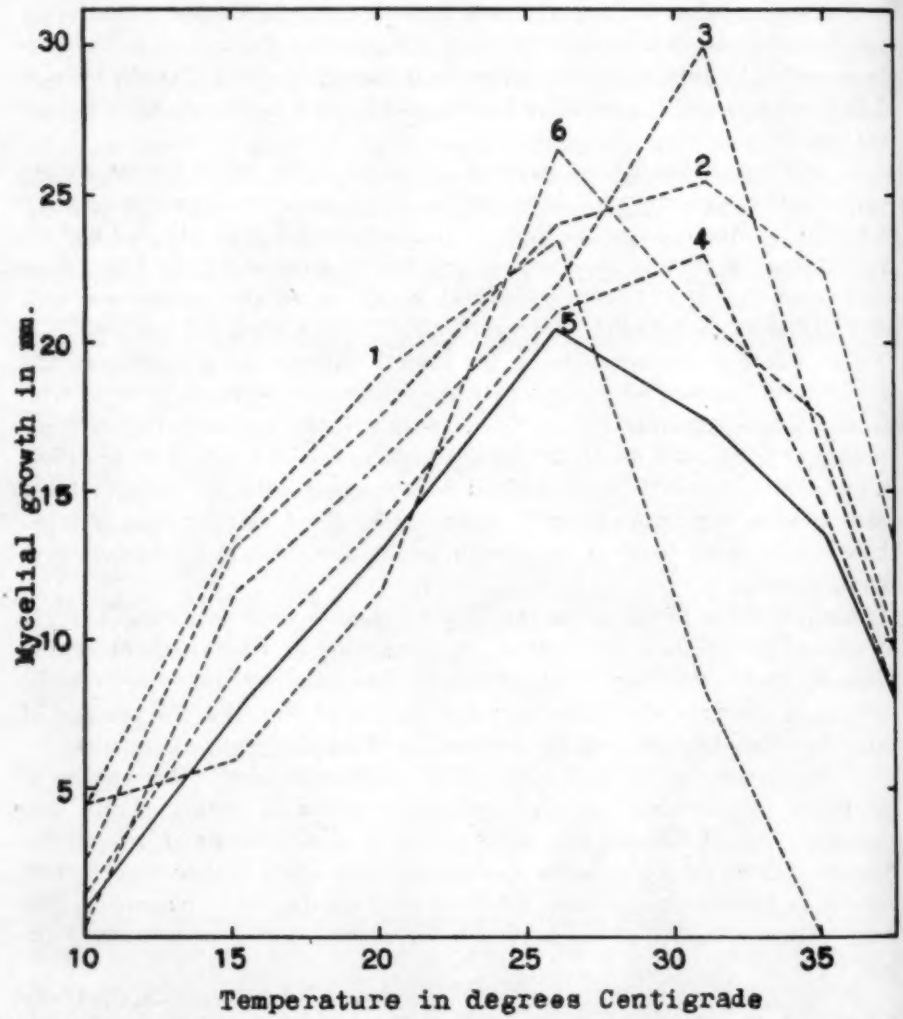


FIG. 8. Graphic interpretation of the temperature-growth relation of 6 *Phytophthora* isolates from data in Table 1. 1) *P. erythroseptica* (*Stockton, California*), 2) *P. drechsleri* (Brentwood, California), 3) *P. parasitica* (Fayetteville, Arkansas), 4) *P. parasitica* (Brentwood, California), 5) *Phytophthora* isolate from tomato foot rot (Stockton, California), 6) *P. drechsleri* (Concord, California).

Two single zoospore cultures were used to study the variability of their progeny, culture No. 494, which had a rapid rate and culture No. 495, which had a slow rate of growth.

From culture No. 494, 23 single zoospore cultures were made. All of them seemed to have a uniform color (white), rate of growth and density of mycelium. From culture No. 495, 41 single zoospore cultures were prepared. Of these 6 had a tan colored and 35 had a white mycelium. The tan cultures, as compared to the white, had a uniformly slower rate of growth and their mycelium was uniformly not as compact.

Survival of the fungus in the soil. Several trials were conducted to determine the period that the pathogen could survive in moist or dry soil in pots containing plants previously inoculated with the pathogen.

The fungus could not be recovered from two pots, containing tomato plants which had been inoculated with the pathogen seven and a half months previously. The tomato plants remained alive up until the soil was tested. Similarly it was not possible to reisolate the pathogen from three pots in which young pepper plants had collapsed through infection, and which were kept dry for six months before their soil was tested.

In one of the trials the fungus was recovered from the soil of two pots in which mature pepper plants were killed after inoculation with the pathogen. The soil of these pots was kept continuously moist and the recovery was made 55 days after inoculation. The watering of these two pots was continued and their soil was tested again for the survival of the pathogen. From one of them it was recovered 95 days and from the other 154 days after the fungus was first introduced into them. Thus it was found that the isolate of *Phytophthora capsici* from tomato foot rot had survived for 5 months in moist soil, which did not sustain a living host.

From the soil of two pots in which tomato plants were inoculated through wounds but were not infected, the fungus was recovered 4 months after inoculation. In the case of two pots in which cowpeas and Lima beans were respectively inoculated along with the tomatoes, and also failed to become infected, the inoculant could not be reisolated a little over 3 months after inoculation.

Discussion. *Phytophthora capsici* Leon. is a pathogen of wide geographical distribution parasitizing in the field several species belonging to the families *Solanaceae* and *Cucurbitaceae*.

Curzi (1927) described a root rot disease of chilies in Italy produced by *Phytophthora hydrophila*. The pathogen also caused a virulent infection of tomato fruits and very young seedlings. Older plants were resistant. The referred binomial is now regarded (Tucker 1931) as a synonym of *P. capsici*. Sarejanni (1936) reported a serious collar rot of chilies in Greece, which was produced by *P. capsici*. The affected plants wilted suddenly and collapsed. Occasional blighting of the fruits was also noted.

Tompkins and Tucker (1937) isolated *P. capsici* from naturally in-

fected honeydew melon fruits, from a planting near Modesto, California. No other part of the host was found to be naturally infected. By inoculating green tomato fruits with this isolate, they obtained lesions typical of buckeye rot. The same authors (1941b) found that the referred species caused on pepper and pumpkin plants a root rot prevalent in San Joaquin Valley near Merced.

According to Kreutzer and Bryant (1946), *P. capsici* was responsible in Colorado for field losses in peppers and eggplants; it was isolated from vines of squash and watermelon, and from fruits of these crops; it was also isolated from fruits of cucumber, honeydew melon, cantaloupe and tomato. They reported epiphytotics of fruit rot of tomatoes in some areas in 1938 and 1939, caused by the fungus. Losses in each of these epiphytoties were estimated by canning companies to be approximately 50 per cent. In 1945, another outbreak of the disease occurred in the Arkansas River Valley. Losses were estimated at from 10 to 25 per cent.

In the present investigation, the isolate of *P. capsici* from tomato foot rot was found to kill young, unwounded tomato plants promptly after inoculation. When plants at the stage before blooming were inoculated without wounding, they developed a dry foot rot and died in from 10 to 15 days. The main root of infected plants often was partially decayed. Mature plants were resistant to the pathogen.

The leaves and the fruits became readily infected. Young fruits were transformed into mummies in about 12 days after inoculation, whereas mature fruits developed a more or less soft rot. The fungus produced sporangia on the surface of infected immature and mature fruits.

The pathogen could kill young, unwounded plants of pepper, pea and Kidney bean. Mature, unwounded pepper and pea plants also collapsed through infection, whereas mature, wounded or unwounded Kidney bean plants developed arrested infection lesions. Immature and mature plants of potato, tobacco, cowpea and Lima bean inoculated through wounds, failed to become infected.

An interesting feature of the isolate was that in winter all zoospores and in summer the great majority of them were non-motile. In contrast to it, isolates of *P. capsici* No. 1661 secured from pepper in California and No. 1727 taken from pepper in New Mexico by Dr. James T. Barrett and given to the author for comparison, produced under similar conditions motile zoospores throughout the year. Also, the two isolates of *P. capsici* from Colorado that Kreutzer and Bryant (1946) worked with, produced motile zoospores.

In a study of the variability of the fungus it was found that the single zoospore cultures varied as to the rate of their mycelial growth in tube slants with potato-dextrose agar, under laboratory temperatures of approxi-

mately 22° C. The single zoospore progeny of a single zoospore culture exhibited a certain amount of variation.

The zoospores were observed to fuse in pairs and occasionally in threes or fours throughout the year. It is significant that Rosenbaum (1917) illustrates swarmspores of *P. arecae* (Colem.) Pethyb., which is a synonym of *P. palmivora* Butler (Tucker 1931), among which 2 are apparently in a state of fusion. Further knowledge about the pairing of zoospores is likely to show that it has an important bearing on the variability and parasitic behavior of the *Phytophthora* species in general.

In trials conducted to determine the period that the pathogen could survive in moist or dry soil, it was demonstrated that it survived for 5 months in moist soil, which did not sustain a living host.

SUMMARY

1. A foot rot of tomato in the canning crop near Stockton, California, yielded cultures of *Phytophthora capsici*.

2. Inoculations with this fungus caused a basal stem rot when the mycelium was added to the soil around the stems of young or half mature unwounded, potted tomato plants.

3. Infected immature and mature tomato fruits developed specific symptoms and signs.

4. The fungus proved pathogenic to unwounded, potted young Kidney bean plants and to young or mature pepper and pea plants.

5. Remarkable features of the pathogen were the non-motility of the majority of its zoospores and their fusion in pairs.

6. The variability of the fungus was studied by making single zoospore cultures. The single zoospore progenies of two single zoospore cultures were studied and differences were found.

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THE NATURE OF ANCIENT WOOD. II. THE STRUCTURE AND PROPERTIES OF WELL PRESERVED TRACHEIDS AND FIBRES

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The present paper primarily aims at studying the fine structure of ancient buried wood with reference to its chemical constituents. The basic fact is that degradative changes in plant tissues of ancient buried woods are characterised by the gradual reduction or modification of the initial cellulose. Such stages of variable degradation in ancient buried wood structure are reported to be those through which plant materials pass during the course of their fossilization or transformation into coal.

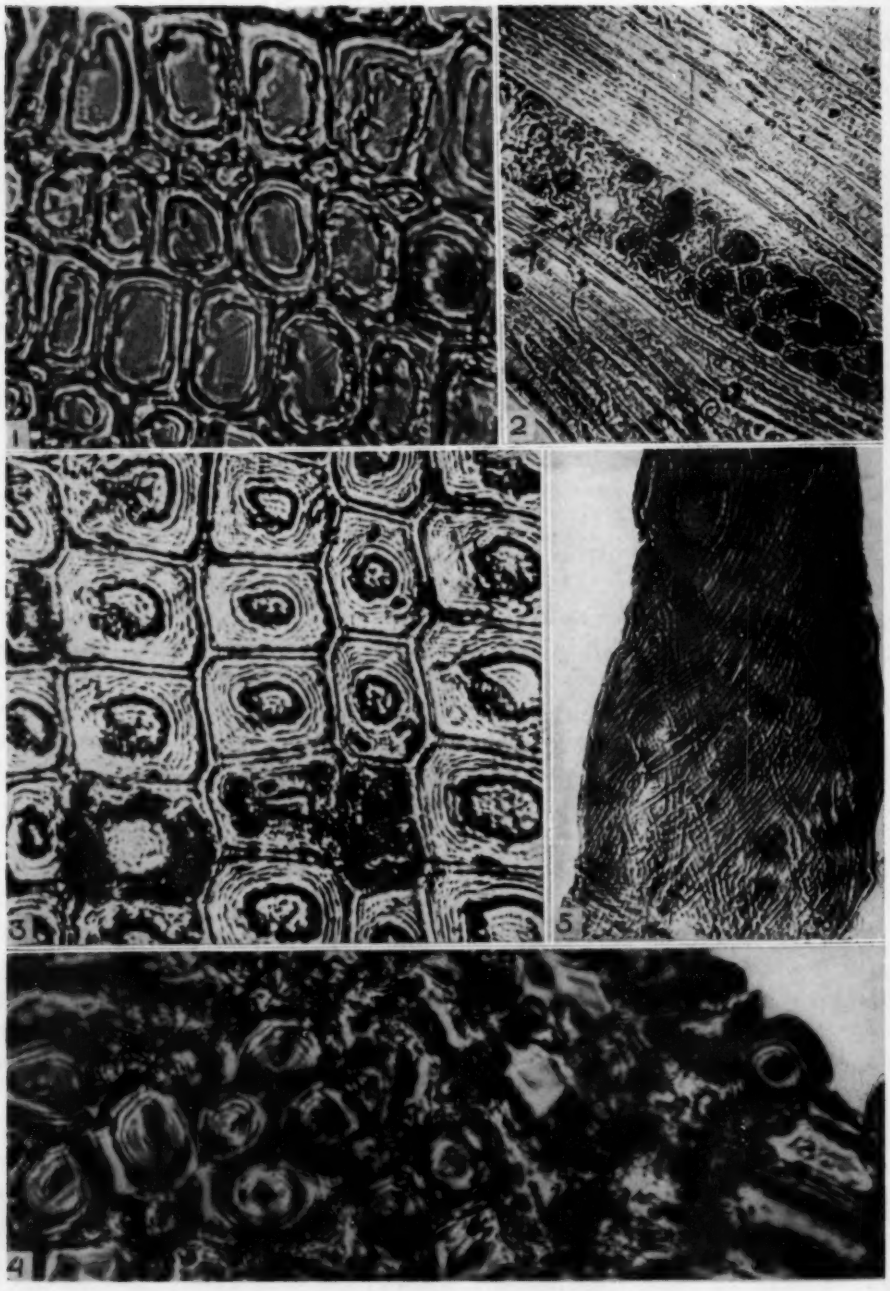
Previous workers have mainly concentrated their attention on correlating the general chemical nature with the gross microscopic physical changes in the cell wall structure of ancient buried wood (Jahn and Harlow 1942; Barghoorn 1949a & b, 1952; Varossieau 1949, 1950). Mitchell and Ritter 1934) and Jahn and Harlow (1942), however, took some notice of the fine structure of the cell wall in such wood. The only detailed work in this line is by Sen (unpub.). This is a step further in studying the structural details (with reference to chemical nature) in materials, collected from widely different sources, which are possibly undergoing fossilization. This aspect of the study has been generally neglected. The present investigation has thus provided an unique opportunity to investigate the variously and gradually degrading cell wall structure in ancient buried wood.

Materials. The materials used in this study were (1) variously degraded ancient buried wood samples of *Pinus sylvestris* Linn. and (2) an ancient buried hardwood sample of Indian origin, namely *Shorea robusta* Gaertn.

The samples of *P. sylvestris* were excavated from piles and foundations, which were exposed when the centre of the city of Rotterdam was destroyed by bombing during the German invasion of Holland in May, 1940. Two such samples were obtained through the courtesy of Dr. W. W. Varossieau of Delft, Netherland. They are (after Varossieau, 1949) sample No. O.K.9. (Oosterkerk, Beam & Joist, 260 years old) and No. 64 (Vlasmarkt sluis, Beam, 600 years old).

During this investigation layers were peeled from the outer surface of the specimens which showed a decreasing deterioration from the external surface towards the internal core of the wood.

Varossieau (1949) has already made microscopic examination of these and other associated softwood samples, and along with Breger (Varossieau and Breger 1951) studied the chemical aspect of degradation.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The hardwood sample *S. robusta* (about 2000 years old) was obtained through the courtesy of Mr. K. Deva of the Department of Archaeology, Central Circle, Patna. This specimen was excavated from the Muryan site of Kumarhar (Pataliputra) in the southern outskirts of the town of Patna (India) at a depth of about 16–17 feet below the surface soil.

Nature of microscopic deterioration. Thin sections of the ancient wood of *P. sylvestris* (O.K.9. & 64) clearly indicate a uniform and consistent sequence of degradative changes in the cell wall layers of the tracheids (fig. 1) (Varossieau 1949) following the general plan laid by Barghoorn (1949a & b). The ancient wood of *S. robusta* reveals the structure of a well preserved porous wood (fig. 2).

A thorough microscopic examination of the ancient wood samples clearly shows the absence of any fungal hyphae and bore holes in the sections of these materials. It may, therefore, be concluded that the Rotterdam materials have possibly suffered from anaerobic or non-biological degradation, whereas the Indian materials might have been slightly affected by non-biological agents. Such types of microscopic degradation may be profitably correlated with their submicroscopic organization.

Microchemical studies. The effect of chlorination and bromination on the cell wall layers of the ancient buried wood is studied carefully by treating the transverse sections in chlorine water and bromine water respectively for different time intervals. Both the sound and ancient materials of *P. sylvestris* behave similarly to chlorination and bromination, showing practically no visible changes in the cell wall layers. The sections are generally cleared after chlorination, and only after prolonged overnight treatment the sections become thinner and brittle, probably due to the removal of the lignin complex.

The secondary wall layers of *S. robusta* are attacked partially after three hours of chlorination, while the vessel walls and ray cells remain unchanged during this time period. When the sections are immersed in chlorine water for about 24 hours, they become thinner, brittle and extremely delicate, due to strong delignification. It was found that bromine water has no effect on these sections. The effect of halogenation confirms the conclusions appearing

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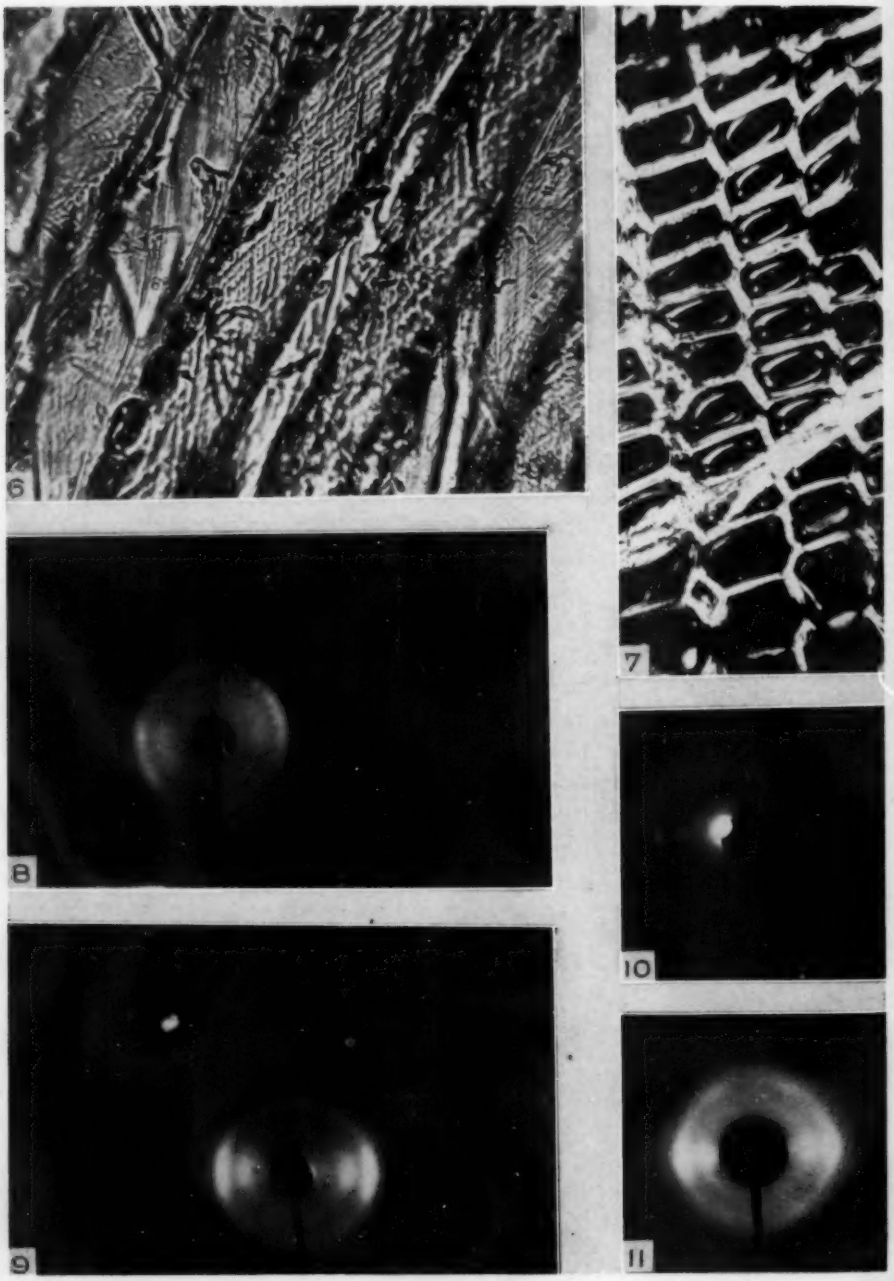
FIG. 1. *Pinus sylvestris* (No. O.K.9.). Transverse section showing nature of deterioration in different layers of the secondary wall. Note that the inner and central layers are mostly attacked. ×1000. FIG. 2. *Shorea robusta*. Longitudinal section showing well preserved cell wall layers. ×320. FIG. 3. *Pinus sylvestris* (No. 64). Transverse section of the late wood after extraction with 72% sulphuric acid. The concentric lignin pattern is prominent in the central layer of the secondary wall. ×1000. FIG. 4. *Shorea robusta*. Transverse section after chlorination for 30 mins. Partial removal of lignin brings out the concentric distribution of cellulose in prominence. ×800. FIG. 5. *Shorea robusta*. Wood fibres cut longitudinally and crushed after swelling in 5% sodium hydroxide solution and followed by staining in 1% aqueous congo red. The apparent cross fibrillar structure is thus revealed. ×530.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later in this paper, that cellulose is well preserved and forms a coherent system with the lignin in these ancient materials as in their modern representatives.

In ancient *P. sylvestris* and *S. robusta* cellulose reacts somewhat similarly to the iodine-sulphuric acid test as in their modern representatives. The reaction is prominent in the central layer of the secondary wall.

The distribution of lignin was also studied. It was found that there is hardly any difference in the intensity of reaction of lignin of different samples to various standard reagents, such as phloroglucinol-hydrochloric acid, and aniline sulphate.

A simple technique for the differential staining of the lignified cell walls, evolved by Lominski and Hutchison (1948) was adopted. A colorless solution of benzidine in glacial ectic acid produces intense orange color in the lignified layers, but causes no visible color change in the cellulosic and other layers. The lamellated appearance of a colorless region alternating with orange colored concentric rings in the central layers of the secondary walls of both ancient and normal wood demonstrates the lignin distribution in these layers. The middle lamella is also stained deep orange.

In conformity with these observations it was found that the secondary wall layers of *P. sylvestris* produce concentric layers of lignin residues (fig. 3) when treated with 72% sulphuric acid following the method of Wardrop and Dadswell (1950). In *S. robusta*, partial removal of lignin after chlorination for 30 minutes brings out the concentric distribution of cellulose in prominence (fig. 4).

Another excellent method of studying the lignin distribution in the cell wall layers can be adopted by staining the sections successively with saturated chlorine water, 3% alcoholic monoethanolamine and 5% aqueous silver nitrate. This technique (Coppick and Fowler 1939) is in fact a modified Tollen's reaction. Following this method the ancient and normal wood of *P. sylvestris* are stained dark brown in the region of the middle lamella which is rich in lignin content, whereas the lightly lignified portions such

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FIG. 6. *Pinus sylvestris* (No. 64). Tangential section of untreated wood showing cross fibrillar structure in the tracheids. This is due to natural and gradual removal of the interspersed lignin. × 320. FIG. 7. *Pinus sylvestris* (No. O.K.9.). Transverse section photographed between crossed Nicols showing bright outer and inner layers and dark central layer of the secondary wall. × 530. FIG. 8. *Pinus sylvestris* (No. O.K.9.). X-ray diffraction photograph using Cu-K target and specimen to film distance being 3 cm. Note the spreading of the equatorial ares. FIG. 9. *Pinus sylvestris* (No. 64). X-ray diffraction photograph using Cu-K target and specimen to film distance being 3 cm. Note that in this case the spreading of the equatorial ares are relatively smaller than those in the sample No. O.K.9., and that the corresponding spots of intensity are sharp. FIG. 10. *Pinus sylvestris* (No. 64—apparently sound core). X-ray diffraction photograph using Cu-K target and specimen to film distance being 3 cm. Note the weak spots of intensity due to the effect of hydrolysis. FIG. 11. *Shorea robusta*. X-ray diffraction photograph using Cu-K target and specimen to film distance being 3 cm. Note the small spreading of the equatorial ares and scattered spots of inorganic matters.

as secondary walls, the color is yellow to amber. The middle lamella, vessel walls, and ray cells of *S. robusta* are stained heavily. The secondary walls of the libriform fibres produce a yellowish colour indicating their low lignin content.

In order to ascertain if the reactant associated is of lignin complex in nature (or lignin-hemicellulose complex), a comparative microchemical study of the action of various lignin and cellulose indicators (phloroglucinol-hydrochloric acid, Schweitzer's reagent, 72% sulphuric acid, etc.) was carried out with successively delignified sections of various ancient wood samples following the schedule of Coppick and Fowler (1939).

It was found that the ancient wood of *P. sylvestris* and its control behave somewhat similarly in the comparative study. The normal samples usually show a greater swelling in sulphuric acid than the ancient ones, possibly due to their high cellulose content. It may also be noted that the intensity of the coloration of the lignin in different staining methods varies inversely to the number of chlorinations. Though both Schweitzer's reagent and sulphuric acid act here as cellulose solvents, the former appears to be less vigorous than the latter.

Ruthenium red (1 : 10,000 aqueous solution), which is reputedly a pectin stain, was used in determining the presence of pectin. The red color in the middde lamella of *P. sylvestris* (ancient) as well as in its control was of similar nature, and thus possibly indicated some preservation of pectin-like substance. The hardwood sample of *S. robusta* also showed the presence of a pectin-like substance in the middle lamella. The possibility that the oxycellulose is responsible for the staining of the middle lamella may be overruled since this region is usually devoid of cellulose-like substances.

Other chemical and physical studies. A comparison of the densities of the different materials clearly showed that with the duration of burial period the value for the densities is found to decrease gradually (table 1). But in the case of sample No. 64 (*P. sylvestris*) a comparatively high density was noted possibly due to the better cellulose preservation (Varossieau and Breger 1951),—since this material is free from any compression.

It was found that with the period of burial the ash content gradually increased (table 2), possibly due to the infiltration of extraneous mineral matters. The comparatively low value of the ash content for specimen No. 64 suggests, in conformity with its high density figure, that the cellulose was better preserved in this 600 year old ancient material. This is evident from the presence of 50% cellulose in this sample (Varossieau and Breger 1951).

A comparative study of the hydrolysis rates in ancient and normal wood was made, using normal hydrochloric acid at ordinary room temperature (26° C.), to find out the general nature of cellulose. The results of loss in weight during hydrolysis in specific time intervals are expressed as percentage of hydrolysis in terms of their initial dry weight (table 2).

TABLE 1

Materials	Density	% of ash	% of sodium hydroxide solubility (1%)	% of alcohol: benzene solubility (1:1)
1. *P. sylvestriss* (normal)	0.7	2	20	5
2. *P. sylvestris* (O.K.9.) (ancient)	0.6	2.35	22	6
3. *P. sylvestris* (64) (ancient)	0.68	2.2	23	5.5
4. *S. robusta* (normal)	0.75	1.86	21.5	8
5. *S. robusta* (ancient)	0.73	1.91	23	5

In the case of *P. sylvestris* the hydrolysis data show that the residual cellulose in the ancient wood is possibly degraded. So far as *S. robusta* is concerned the situation is, however, complicated and, therefore, any comment upon it should be reserved for the present.

The solubility rates and the quantity of extracts from all these ancient materials, with reference to their control, were also determined using different solvents, such as 1% sodium hydroxide solution and alcohol-benzene (1:1) mixture. Here again the percentage of the loss for a given time period is calculated on the basis of the initial dry weight (100–110° C.) of the materials. As generally expected in the ancient-wood the lignin solvent acts vigorously which is an indication of decay (Freeman 1946). The total alcohol-benzene extracts are less in the case of ancient wood. Possibly the substances in the extracts decompose very slowly.

TABLE 2. *Hydrolysis of normal and ancient wood samples in normal hydrochloric acid at room temperature 26° C.*

Materials	30 min.	60 min.	120 min.
1. *P. sylvestris* (normal	11%	12%	12.5%
2. *P. sylvestris* (O.K.9.) (ancient)	15%	16.3%	17%
3. *P. sylvestris* (64) (ancient)	12%	13.3%	14%
4. *S. robusta* (normal)	12%	14%	15%
5. *S. robusta* (ancient)	8%	9.5%	10%

Structure of ancient tracheids and fibres. Studies on fibrillization of ancient tracheids and fibres. The fibrillar orientation was observed by gently crushing the isolated chromic macerated ancient tracheids and fibres, followed by swelling in 5% sodium hydroxide solution and staining in 1% congo red (Wardrop and Dadswell 1948). The angle of spiral is 30–32° in ancient *S. robusta* (fig. 5), and is 20° in ancient *P. sylvestris* (O.K.9.). In one of the samples (No. 64) of *P. sylvestris* the cross fibrillar structure appeared without any treatment (fig. 6). This is possibly due to the gradual removal of some interspersed lignin during burial. The fibrillar orientation generally indicates the presence of variously oriented cellulose micelles in the ancient wood samples.

Optical nature of ancient tracheids and fibres. The typical cell wall in the wood of higher plants consists of (1) the primary wall and (2) the secondary wall of three morphologically and optically distinct layers or lamellae. These three layers of the secondary wall are (a) a relatively thin outer layer, (b) comparatively thick central layer, and (c) a thin inner layer lining the lumen.

In the transverse sections of both normal and ancient wood the primary wall and the inner and outer layers of the secondary wall exhibit pronounced birefringence in contrast to the broad isotropic central layer (fig. 7). This optical heterogeneity is due, partially, to the differences in the micellar arrangement within the wall layers (Wardrop and Dadswell 1948). Therefore, it may be qualitatively estimated that in the ancient wood samples, as in their modern representatives, the micellar arrangement in the central layer is in relatively steep spirals compared to those in the primary

TABLE 3

Materials	Range of striation direction in 100 fibres	Range of major extinction position in 100 fibres
1. *S. robusta* (normal	6–12	5–11
2. *S. robusta* (ancient)	1–7	0–5

wall and inner and outer secondary layers. Similarly Mitchell and Ritter (1934) observed preferred orientation in some ancient buried coniferous wood mined from the miocene auriferous gravels of California. The cellulose in all these samples is obviously better preserved than in many other cases noted before (Jahn and Harlow 1942, Sen unpub.).

The visible striation directions in thin longitudinal sections of both modern and ancient wood of *S. robusta* have also been measured. The major extinction position of the corresponding fibre was also noted to compare with the data obtained from the striation direction measurements. It may be mentioned that the values for striation direction and major extinction position for the same fibre do not vary greatly and are generally corroborative (table 3); this would argue strongly in favor of the existence of only one set of cellulose chains (Kundu and Preston 1940). Surprisingly, however, the cellulose in the ancient materials appears to be relatively well oriented.

X-ray studies of ancient wood. The samples for the X-ray studies were tangential chips (0.5 mm. thick) of the sapwood. These were first thoroughly washed in water and finally dried in air. The diffraction patterns were obtained by using Cu-K radiation. The specimen was placed at a distance of 3 cm. from the film, and the exposure continued for about 5 hours.

In the ancient buried wood of *P. sylvestris* (O.K.9. and 64) the micelles were found to be in preferred orientation (figs. 8–9). Since photometric

measurements could not be obtained, the spreading of the equatorial arcs in the X-ray diffraction photographs has been studied only for comparative purposes. In the sample No. 64 the relatively sharp spots of intensity and less spreading of the equatorial arcs show that the cellulose is more oriented and crystalline as compared to that in the sample No. O.K.9. (figs. 8–9). It is difficult to suggest whether there is also any tendency to disorientation due to burial. The small spreading of the equatorial arcs in the apparently sound core of the ancient *P. sylvestris* indicates the presence of highly oriented cellulose. The apparent loss of intensity of the spots is due to hydrolysis in the laboratory (fig. 10). In sample No. O.K.9. the presence of some background scattering may be due to the accumulation of extraneous matter and lignin (fig. 8).

The ancient dicotyledonous wood of *S. robusta* produces preferred X-ray diagram showing sharp spots of intensities (fig. 11). This material has possibly not suffered any degradation. The micellar angle is small with respect to the longitudinal fibre axis. The scattered background spots may be due to the accumulation of inorganic matter. It may be suggested that a tendency for "biological fixation" in the sense adopted by Barghoorn (1949a) is apparent in this and in sample No. O.K.9. of *P. sylvestris* (figs. 8, 11).

Discussion. Throughout this paper an attempt has been made to demonstrate the nature of the fine structure in anaerobically or non-biologically degraded ancient buried wood by employing different physical and chemical methods, such as X-rays, polarized light, microchemical reagents, chemical solvents, etc. The preservation of the structure, as expected, obviously depends upon the cellulose, the principal cell wall constituent, and its intrinsic nature.

The distribution of the cell wall constituents in the ancient buried materials is interesting. For studying the location of lignin and its relation to the other cell wall constituents differing techniques have been successfully adopted. It has been shown that the lignin may remain practically unmodified in ancient wood during its long period of burial. The distribution patterns of lignin in different materials was found to be closely comparable to those in their modern representatives. The conspicuous red color produced by the ruthenium red in the middle lamella also demonstrates the possibility of the preservation of pectin-like substance in these excavated materials, which can only be removed after hydrolysis in the 10% hydrochloric acid at about 90° C. This situation may be compared with that in the normal materials.

Sections of the ancient wood of *P. sylvestris* react relatively less vigorously to the known cellulose solvents (Schweitzer's reagent, sulphuric acid, etc.) unlike their modern representatives. The reason is obvious. It was found that the lignin solvent acts more or less vigorously on all the de-

graded wood samples of *P. sylvestris* and *S. robusta*. This is possibly due to the simplification of lignin molecules and gradual removal or modification of cellulose. The action of normal hydrochloric acid on ancient wood samples of *P. sylvestris* also shows the relative degradative nature of the cellulose rather qualitatively.

The density of the ancient wood samples usually decreased with the age. This was expected due to the gradual removal of the cellulosic structures from the cell wall. In one case (*P. sylvestris*—No. 64), however, the density of the ancient wood was found to be almost like that of its control. This is due to the preservation of cellulose. Since the ash is not unusually high, this situation can not be due to the accumulation of inorganic matters.

In ancient buried wood the cellulose chains are variously oriented in the different layers of the secondary wall with reference to the major longitudinal axes of the tracheids or fibres. The samples of ancient wood of *P. sylvestris* (O.K.9. & 64) as compared with the normal, exhibit weak birefringence. But the ancient wood of *S. robusta* and its control gave almost similar birefringence. It was also found that the major extinction position data usually do not differ very widely from those of the visible striation directions in the fibres of *S. robusta*. Thus the data obtained clearly show that the organization of the cellulosic structural framework has been variously preserved in the different samples of the ancient buried wood.

In the light of X-ray diffraction photographs, the cellulose is *P. sylvestris* (O.K.9., 64 and the apparently sound core of 64) and *S. robusta* is also found to be quite well oriented. The background scattering when apparent, is probably due to the lignin and accumulation of the extraneous inorganic mineral matters in the ancient wood. Generally the spots of intensities, excepting in the sound core of sample No. 64 which has been artificially hydrolyzed in the laboratory, are sharp. This indicates the presence of good amount of crystalline cellulose. The loss of cellulose in the apparently sound core of *P. sylvestris* by heating in water under pressure results in the weak intensity spots of the X-ray diffraction diagram. That the heating of wood with water under pressure causes variable loss of carbohydrates (Overbeck and Muller—see Hagglund 1951) is now an established fact.

It may be concluded from the available data that the basic anatomical organization is dependent upon the presence of sufficiently well oriented crystalline cellulose, i.e., the fine structural framework of the cell wall. The sample No. O.K.9. is microscopically most degraded. This is also revealed to some extent in the X-ray diffraction photograph. The other materials may also be similarly compared. Thus a correlation exists between microscopic and submicroscopic organization.

It is evident from a survey of these ancient materials that the extent of degradation is not directly related to age alone. Among the ancient

wood samples of *P. sylvestris* (O.K.9. & 64) the older (No. 64) is relatively well preserved. Surprisingly the oldest of the ancient wood (*S. robusta*—2000 years old) has been found to be the best preserved. The experimental data indicate clearly that the cellulose is almost intact in this wood. Possibly the intrinsic properties of the different wall layers, the nature of lignin protection, the relatively high density of the wood, the nature of burial and various other environmental factors, such as temperature and pressure, all have a cumulative effect on the extent of degradation.

Recently Varossieau and Breger (1951) studied the fate of lignin in the ancient buried wood in the ultimate production of humic substances. Although there are disputed theories about the origin of coal, present workers in this field are unanimous in their opinion that the cellulose is generally destroyed. Consequently there is an apparent increase in the lignin content, which is possibly modified into humus. It has also been pointed out by Barghoorn (1949a & b) and Varossieau (1949) that the degraded ancient wood samples when compared with fossils and subfossils clearly indicate a striking similarity in their microscopic cell wall structures, such as in their granular appearance, dark color of their ray cell contents, etc. From his detailed investigations, Varossieau (1949) concluded that "The characteristic changes in the cell walls of the wood from Rotterdam pilings are to be considered as identical with the first of a series of changes which wood undergoes during conversion to coal or during petrifaction."

It has also been observed by Barghoorn (1949a) that mineralization of plant remains may be effected when cellulose still persists. This may be one of the steps for "biological fixation" of the plant materials undergoing fossilization. In the X-ray diffraction photograph of the ancient wood of *S. robusta* the presence of scattered inorganic materials indicates this tendency. The increasing ash content in the ancient buried wood also adds evidences to such possibilities.

SUMMARY

1. The cell wall structure of tracheids and fibres of some ancient buried wood samples collected from several sources were studied. These include anaerobically or non-biologically degraded ancient buried wood of different ages, namely *Pinus sylvestris* (sample Nos. O.K.9. & 64 from Rotterdam), and *Shorea robusta* (from Muryan site, Pataliputra, India).

2. The extent of deterioration exhibited by all these ancient woods is not related to the age alone. The nature of preservation obviously depends chiefly upon the intrinsic properties of the wood, environmental conditions, etc.

3. In the softwood samples the microscopic degradation of the cell wall lamellae follows a graded sequence as outlined by Barghoorn (1949a). Sur-

prisingly the hardwood sample, which is oldest in age, was found to be almost free from any degradation.

4. It was found from solubility tests and microchemical studies that in the ancient buried wood a large amount of original lignin was always present. The distribution of lignin was found to be similar in both ancient wood and its control. A pectin-like substance has also been noted.

5. The X-ray and other optical evidences indicated that in all these ancient materials the cellulose is variously oriented. This together with other evidences clearly indicate the presence of substantial amounts of resistant cellulose in these materials.

6. A gradual increase in the ash content, and the presence of scattered spots in one of the X-ray diffraction photographs, are due to the accumulation of inorganic matters. This possibly initiates "biological fixation" of structures in the course of fossilization.

7. Thus the entrancing beauty in the ancient plant life going down to the low limits of visibility is revealed. The role of such materials in fossilization has appeared to be no less interesting.

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BULLETIN OF THE TORREY BOTANICAL CLUB
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THE FOUR SUBFAMILIES OF THE GRAMINEAE[1]

Alan A. Beetle

Most grass lovers living in temperate regions see only the end results of lines which have pursued divergent evolution for long periods. There seems to be general agreement among agrostologists that if representatives of our primitive grasses have survived it has been in the tropics. From time to time a new genus or species has been described and a note attached indicating primitive characters and uncertain relationships (as Bews put it, "of phylogenetic rather than ecological or economic interest"). The number of these has now reached such formidable proportions that a new look at all the evidence seems in order.

Among these genera showing certain primitive features (excepting for the moment the tribes of the Bambusoideae) are *Streptochaeta* (2 species), *Anomochloa* (1 species), *Pharus* (6 species), *Leptasis* (4 species), *Pariana* (32 species), *Olyra* (7 species), *Cryptochloa* (4 species), *Mniochloa* (2 species, *Diandrolyra* (1 species), *Raddia* (10 species), *Lithachne* (2 species), and *Buergersiochloa* (1 species). The species of these genera have in common a leaf blade narrowed to a short petiole-like base, prominent crossnerves in addition to the main nerves, and, as pointed out by Page (1947), a special kind of tissue composed of greatly enlarged cells which collapse at maturity. Regardless of whether these broad, tropical rain forest leaves are primitive, there are two other morphological features which support such a theory, i.e., the presence of well-developed lodicules, and extra stamens (more than 3, often 6, or many). Bews (1928) made much of their tropical rain forest habitat as evidence of their primitiveness, and one likes to forget Bessey's suggestion that woody plants are more primitive than herbaceous, and try to trace our temperate grass flora from these herbaceous perennials rather than from the strange maze of taxonomically difficult and woody bamboos.

Again, turning to the Besseyan dicta for a notion of what is primitive and what is advanced, it becomes clear that many-flowered spikelets in open panicles of the Festuceae are considered more primitive than one-flowered spikelets, for the perfect florets of the Festuceae are considered more primitive than the imperfect florets of monoecious plants. From the point of view of practical phylogeny it would be convenient to have a "primitive grass" that could lead toward the perfect, many-flowered types of the Festuceae.

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All of the above-mentioned genera are one-flowered, in rather specialized spike-like or racemose inflorescences, and only two of them have perfect florets (*Streptochaeta* and *Anomochloa*). Furthermore, the articulation is always below the glumes (the exception rather than the rule in the Festuceae).

From the point of view of distribution the genera are again a disappointment. In family after family of the Angiosperms the most primitive types have been traced back to the southern half of the Asiatic continent. But of these so-called primitive grasses 10 genera and 85 species are New World (including the two genera with perfect florets), while only six species representing three genera are Old World. Obviously, if the New World types are clung to as representing the most primitive grasses, one would have to think about an American center of origin for the grass family.

The primitive characters of the Pharoideae may be listed as (1) well-developed lodicules, (2) extra stamens, (3) perfect spikelets, (4) herbaceous perennials, (5) tropical forest distribution, (6) petiolate leaf with special cells and special venation. It is perhaps significant that all the above characters may be found in the Bambusoideae (particularly in the genera of the Arundinarinae), and that here one finds such additional primitive characters as (1) articulate leaves, (2) woody stems, (3) awnless glumes and lemmas, (4) lemma-like glumes, (5) many-flowered spikelets.

The conclusion here drawn is that the genera of the Pharoideae had an independent origin from the Bambusoideae (surely the most primitive grasses) and that the recognition of four subfamilies will represent the phylogeny of the grasses more clearly than do the two subfamilies usually recognized.

Family Poaceae (Gramineae)
- Subfamily I. Bambusoideae
  - Tribes Dendrocalaminae and Melocanninae (Old World)
  - Tribes Bambusinae, Arundinarinae, and Puellinae (Old and New Worlds)
- Subfamily II. Pharoideae
  - Tribes Streptochaeteae (*Streptochaeta*)
  - Anomochloae (*Anomochloa*)
  - Phareae (*Pharus, Leptasis*)
  - Parianeae (*Pariana*)
  - Olyreae (*Olyra, Cryptochloa, Mniochloa, Diandrolyra, Raddia, Lithachne, Buergersiochloa, Froesiochloa*)
- Subfamily III. Festucoideae
- Subfamily IV. Panicoideae

This treatment strengthens the naturalness and the distinctness of the subfamilies Festucoideae and Panicoideae. It also resolves much of the confusion over the placement of the various tribes of the Pharoideae.

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THE MORPHOGENETIC EFFECTS OF VARIOUS CHEMICALS ON THE GEMMAE OF *LUNULARIA*[1]

CARL D. LARUE AND S. NARAYANASWAMI[2]

From the time of Voechting (1885) considerable attention has been given to regeneration and vegetative reproduction in the thallus liverworts. In recent years Voth (1941, 1943 and 1945) has made extensive studies on the mineral nutrition of thalli of *Marchantia,* and Voth and Hamner (1940) have studied the effects of nutrient supply and photoperiod.

In the meantime, the gemmae have been the subject of numerous investigations. Fitting (1942) has presented a series of studies on the question of the development of dorsiventrality of liverwort thalli, while Halbsguth (1936) has made a thorough investigation of the developmental morphology of the thalli from the gemmae. Later Halbsguth (1953) and Halbsguth and Kohlenbach (1953) extended their studies to investigate the effects of growth substances on the symmetry of young thalli produced by the treated gemmae. Similar studies have been made by Rousseau (1951) especially on the effect of chemical substances on the growth of gemmae of *Marchantia.* Nagai (1919) secured interesting forms of thalli from gemmae of *Marchantia* which had been plasmolysed but apparently no one has extended this work and until the beginning of the research reported here, no one has made a general study of the possibilities of morphological variations which may be induced by the application of various stimuli to the gemmae.

We began work on gemmae because, as we looked over the world of green plants, it appeared that these structures should lend themselves admirably to studies in experimental morphology. They are large enough to be handled readily, but are not large enough to take up an undue amount of space. They are green plants and do not need to be supplied with any external source of sugar, and therefore, do not need to be grown in sterile culture. In spite of the fact that many liverworts contain fungus symbionts, we have found the gemmae can be sterilized and grown in sterile culture, but it is much simpler to grow them on carbohydrate-free media as we have done throughout this study. No expensive or unusual type of equipment is needed. Thalli grow out rapidly from the gemmae and results

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[2] Fulbright Fellow at the University of Michigan, from the Department of Botany, University of Delhi, India, 1953–1954.

may be secured in a matter of weeks instead of months as with many other types of material. What we did not know in advance was that the gemmae are extraordinarily sensitive to changes in a number of environmental factors. Beyond our hopes, they have proved especially good subjects for experimentation of a number of kinds.

Methods. Gemmae have been lifted out of the gemmae cups and put on filter paper in petri dishes. The filter paper has then been saturated with a solution of the chemical to be tested. After a period of 24 or 48 hours the gemmae have been transferred to fresh filter paper saturated with the mineral nutrients of White's (1943) solution. The petri dishes were then placed in a greenhouse on the north wall of our laboratory. More nutrient solution was added as needed, and the cultures were examined at regular intervals. Some volatile materials such as acenaphthene and naphthalene have been used by simply placing a crystal of the material in a petri dish in which the gemmae are lying on filter paper saturated with a solution of mineral nutrients.

A variation of the method consisted of placing the gemmae on a filter paper saturated with the test solution, then folding one half the filter paper over on the gemmae so that both upper and lower surfaces of the gemmae were in contact with the wet filter paper.

Some experiments have been performed in which the gemmae have been grown continuously on paper saturated with a solution of the test chemical. Also, as will be noted, some gemmae were treated and then grown on a klinostat. The controls used in these tests were grown on filter paper saturated with the mineral nutrients of White's solution. Hereafter when we refer to White's solution, it should be understood that we mean the mineral nutrients of White's solution.

Chemicals used. Although we were interested in the morphology rather than genetical effects, we used several substances known to induce polyploidy, at least in higher plants, because these have been known to cause rather profound morphological changes. Growth hormones also were rather obvious choices for study. The remaining substances were simply chemicals which were on our shelves and were chosen almost at random. The greater number of the substances were readily soluble in water, but a few were not very soluble and some volatile chemicals were used, as well as a gas or two.

Results. A new type of branching. The first notable effect of the treatments of the gemmae was a totally unexpected one. It is a new type of branching; one unknown elsewhere in nature so far as we can ascertain (LaRue and Narayanaswami 1954). Since our report of this kind of branching, we have learned that Förster (1921) reported this branching as an abnormal type, and both Halbsguth (1936) and Fitting (1942) have studied it.

Normally, lobes grow out from each of the two growing points of a

gemma. Each of these lobes grows into a thallus with a single growing point in the middle of its tip which contains an apical cell which cuts off cells from two faces and broadens out the thallus. When the thallus becomes sufficiently broad, new apical cells are formed and bifurcation of the tip occurs.

The new type of branching by contrast, occurs at right angles to the regular type of branching which has just been described and forms two lobes, one above the other. Both lobes are parallel to the expanded surface of the thallus (fig. 1). An upper and a lower lobe are produced by this

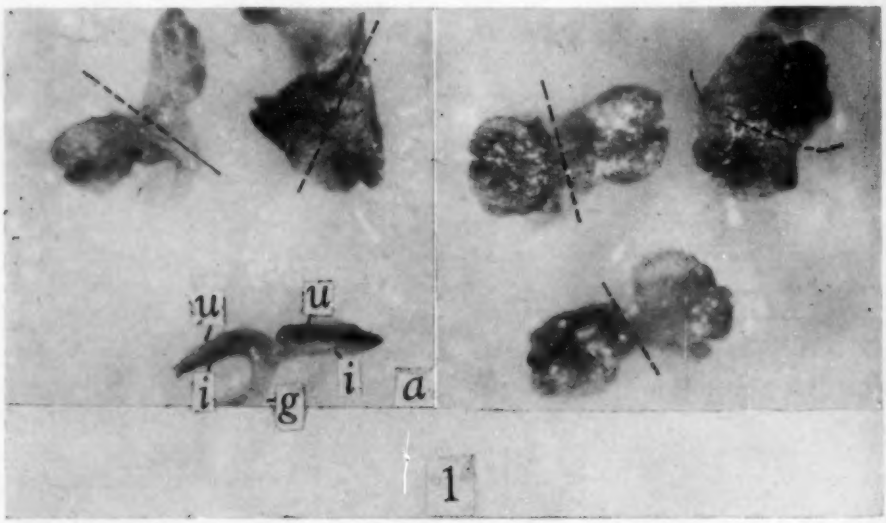


FIG. 1. Young thalli of *Lunularia* showing splitting. The dotted lines show the planes of splitting. *a*—shows a thallus which has formed two equal lobes after splitting. (Abbrev.: *g*, body of the gemma; *u*, upper surface; *l*, lower surface).

process which we have become accustomed to refer to, in the course of this study, as *splitting* (fig. 2 *a-e*). This is a very descriptive term and the tip of the thallus with its two lobes looks as though it had been split.

It seems that almost as soon as the split occurs the thallus begins to overcome the effect, usually by the unequal growth of the two lobes. The lower lobe usually grows more rapidly than the upper and apparently exercises a dominance over it (fig. 2 *d–f*). Later the evidence of the *split* is shown only by a ridge in the thallus or sometimes a flap or a ligule-like lobe or knob is all that is left of the upper lobe (fig. 2 *i, j*). Rarely an upper lobe swings off to one side so that the two lobes come to lie side by side. Occasionally the upper lobe will grow rapidly and the lower lobe will be suppressed and overgrown (fig. 2 *g–i*). After a few weeks it is difficult to find more than very slight traces of this type of branching.

Occurrence of branching of the new type. No evidence has been found of splitting in greenhouse growths of *Lunularia* and *Marchantia* which contained thousands of plants, but we have found a few splits in our controls. The gemmae in these controls may not be entirely normal, since they have been transferred from the gemmae cups to filter paper soaked with White's solution in petri dishes, and have been grown under these conditions. Aside from these few control plants, this type of branching has not been found occurring naturally anywhere in the plant kingdom, although Labouriau (1952) has secured a somewhat similar effect by treating leaves of ferns with growth hormones.

Thus far, splitting has been produced only from gemmae of *Marchantia* and *Lunularia*. Because of the greater supply available to us, the greater part of this study was carried out with *Lunularia*.

Factors which induce splitting. Almost all the chemicals which have been tested have produced the new type of branching to a greater or lesser degree. Among those which are active are the following: acenaphthene, actidione, caffeine, chromic acid, colchicine, ether, naphthalene, parathion, and sodium cacodylate. Figure 2 *a–j* is illustrative of the nature of splitting apparently induced by treatment with colchicine, 1×10^{-2}M, 1×10^{-3}M and 1×10^{-4}M for 48 hours. Caffeine, sodium cacodylate, naphthalene, actidione and parathion have also proved effective in causing a similar phenomenon (figs. 3 *a–d;* 4 *a–c* and 5).

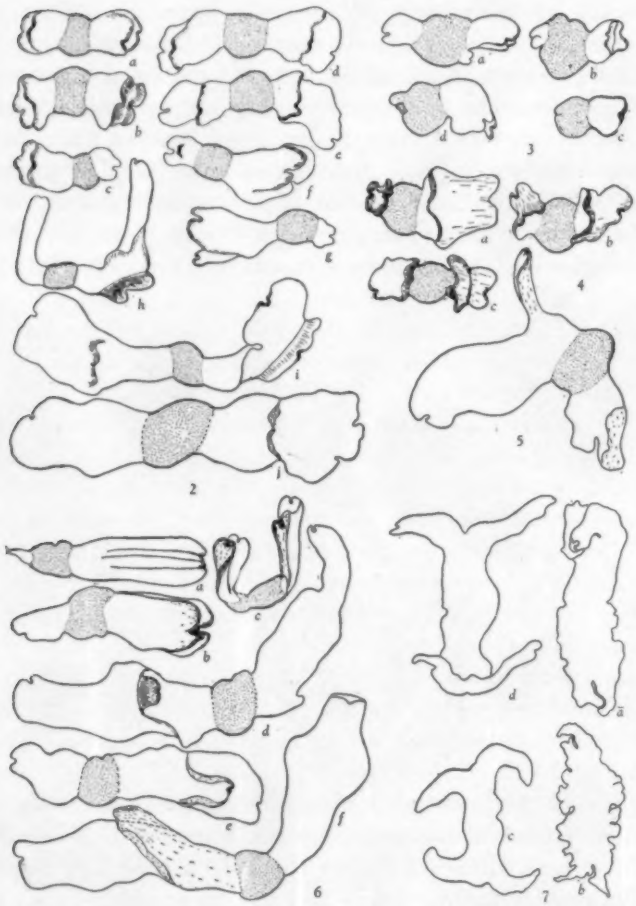
Thus far we have seen no *splitting* due to treatments with beta-methyl umbelliferone, benzoguanimine, maleic hydrazide, coumarin, thiourea, IAA, NAA, IBA and 2,4-D.

In addition to treatment with chemicals, *splitting* has been induced by plasmolysis with various solutions; by mutilation; and by irradiation with ultraviolet rays and with rays from a cobalt-60 source. The results of these latter factors require further study and will be presented elsewhere.

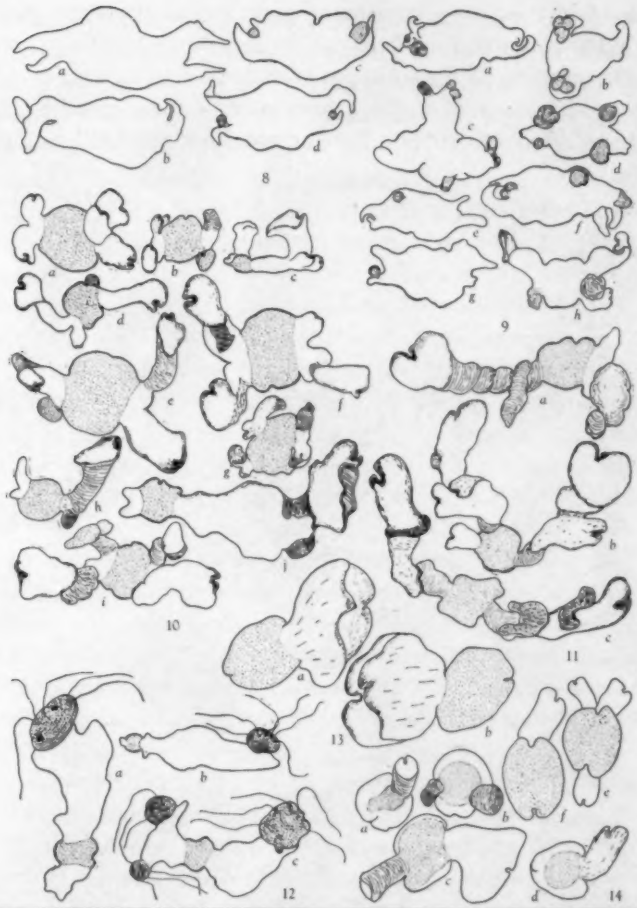
Orientation of the new type of branching. We have described the *splitting* as producing an upper and a lower lobe, and at once, the question arises as to the orientation of these lobes. Klinostat tests were run with gemmae which had been treated with different chemicals. In all trials, it was found that the splitting always occurred parallel to the flat axis of the thallus, without any regard to the orientation of that thallus in relation to gravitational pull. *Splitting* is able to take place in any position in which the gemmae have been placed (fig. 6 *a–f*).

Splitting is restricted to growths from gemmae. Some trials have been made with tips of mature thalli, but in no adult specimen has *splitting* been observed.

Variation in shapes produced in response to treatment with various chemicals. Aside from the new type of branching almost all of the

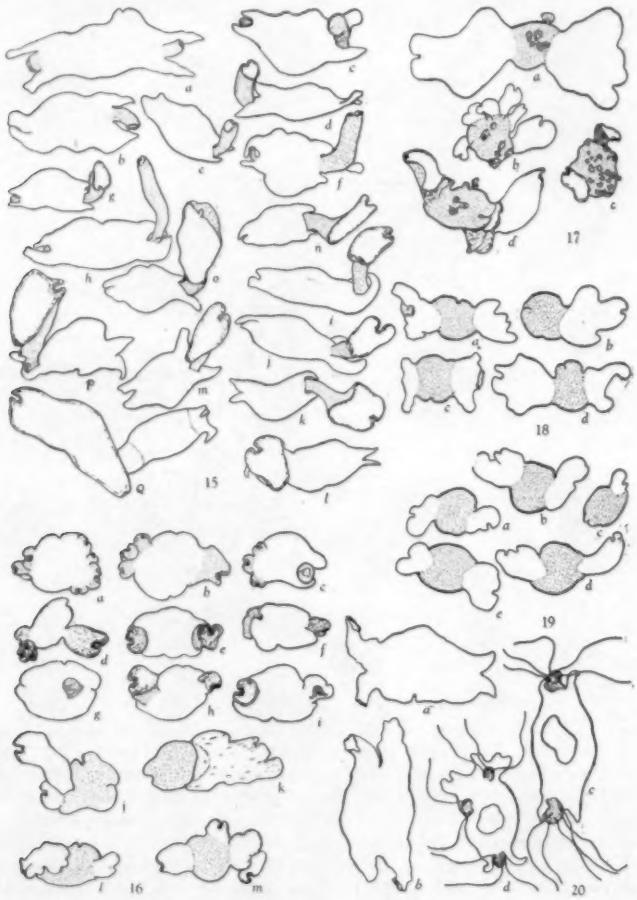


FIGS. 2–37 were drawn from a Bausch and Lomb wide-field binocular microscope with the aid of a camera lucida. FIG. 2, a–j. Early and late stages in development of gemmae showing splitting induced by treatment with colchicine, 1×10^{-2} M, and 1×10^{-3} M for 48 hours. × 10. FIG. 3 a–d. One-week old gemmae showing splitting induced by naphthalene. × 6. FIG. 4, a–c. Same as above, with actidione. × 6. FIG. 5. One-month old gemmae showing narrower upper and broader lower lobes of a split induced with parathione. × 6. FIG. 6 a–f. Stages in development of colchicine-treated gemmae on klinostat; note lower lobe has outgrown the upper *d*, *e*, and *f*. Three-weeks old. × 6. FIG. 7, a–d. One-week old gemmae treated with 2,4-D, 0.1%; note "Buffalo-horn" type of lobes in *c* and *d*, and fringed margins in *a* and *b*. × 6. FIG. 8, a–d. Effects of 2,4-D. *a* and *b* show inhibition of growing initials with subsequent growth of bodies of gemmae and development of points or lobes in *c* and *d*. × 6. FIG. 9. Effects of 2,4-D. *a–h*, gemmae bearing buds between points, on points, and on bodies of gemmae. × 6. FIG. 10. Effects of 2,4-D *a–j*, further development of buds and upright lobes bearing "feet." × 6. FIG. 11. Effects of 2,4-D. *a–c*, segmented type of thalli. × 6. FIG. 12. Effects of 2,4-D. *a–c*, thalli grown on White's solution, later treated with 2,4-D: Note the abnormally large spherical buds and rhizoids. × 6. FIG. 13. Effects of TIBA, 1×10^{-4} M. Ten-day old gemmae showing splitting of the broad, fan-shaped thalli at tip (*a* and *b*); note inhibition of growth from the second growing point. FIG. 14. Effects of dowicide, 0.5 gm./1/ for 5 minutes. *a-c*, gemmae bearing short, succulent, cylindrical lobes;



d–f, gemmae with thick, flat thalli from side and lower surfaces. The unstippled area shows dead portion of gemma. FIG. 15, a-q. Developmental stages of gemmae treated with acenaphthene. ×6. FIG. 16, a–m. Same, with prolonged treatment with sodium cacodylate, 1×10^{-2} M. ×6. FIG. 17, a-d. Gemmae bearing supernumerary buds and thalli induced by treatment with actidione, 0.01 gm./1. ×6. FIG. 18, a-d. Two-week old gemmae treated with parathion, 1 gm/1. ×6. FIG. 19, a–e. One-week old gemmae exposed to vapor of naphthalene. ×6. FIG. 20. Effects of maleic hydrazide, 0.5% and 1%. *a* and *b* show formation of buds on wounding. FIG. 21, *a–d*. Thallus shapes. Gemmae treated wtih benzoguanamine, 0.1 gm/1. ×6.. FIG. 22, a–c. Same, formalin, 0.01%. ×6. FIG. 23, a–f. Same, coumarin, 0.5 gm/1. ×6. FIG. 24, a–g. Same, ether. ×6. FIG. 25, a–e Same, ether. ×6. FIG. 26, a–g. Same, phemerol, dilute solution. ×6. FIG. 27, a–c. Same, diphenylamine, 0.1 gm./1. ×10. FIG. 28, a–h. Stages in development of gemmae treated with diphenylamine, 0.1 gm./1. ×10. FIG. 29, a–c. Same, with thiourea, 0.1 gm./1, six weeks. FIG. 30, a–f. Same, on prolonged treatment (two-weeks) with colchicine, 1×10^{-2} M. ×10. FIG. 31, a–f. Gemmae treated with colchicine and grown in diffuse light on klinostat. ×6. FIG. 32, a–c. Same as above, and grown in greenhouse. ×6. FIG. 33. a–c. Gemmae treated with naphthalene and grown in diffuse light on klinostat, later transferred to greenhouse. ×6. FIG. 34, a–c. Same as above, with sodium cacodylate. ×6. FIG. 35, a–c. Same as above, with acenaphthene. ×6. FIG. 36, a–c. Same as above, with caffeine. ×6. FIG. 37, a–b, Control, on White's solution, treated as above. ×6.

chemicals used in treating the gemmae gave a characteristic effect. It is remarkable that such a definite effect can be caused by treatment with a given substance. Description of these shapes is difficult and camera lucida sketches are used to convey an idea of the appearance of these growths. Typical examples were selected as subjects for camera lucida drawings and care was



taken to see that all illustrative material was characteristic of the lot under study.

2,4-D, 0.1%. The gemmae treated with this substance elongate greatly and long pointed auricles grow out from each end of a gemma (figs. 7 *a–d*, 8 *a–d*). The two auricles at one end of a gemma tend to approach (fig. 7 *a, b*) and sometimes wrap around each other (fig. 8 *b*). More rarely the auricles diverge widely (fig. 7 *c, d*) and remind one strongly of the reflexed horns of a water buffalo. Later, dome-shaped buds appear between the

auricles, and almost as often on the auricles as between them (fig. 9 *a–h*). Eventually, buds may arise almost anywhere on the upper surfaces of the gemmae and often on the lower ones too. Some buds become greatly enlarged and spherical (fig. 9 *d, f*). These are thickly covered with large rhizoids. From the dome-shaped buds, stalked lobes grow out which are often cylindrical and upright, though sometimes they are neither cylindrical nor upright. These never form a normal thallus but instead they become expanded on top to form fantastic structures (fig. 10 *c–h*). The lobes grow out, become checked, bud out again and repeat this process until thalli are formed which have a curious arachnoid appearance (fig. 11 *a–c*), and are vastly unlike any thallus of *Lunularia* ever encountered in nature. The tips of the lobes may become spread out to form a cup, (fig. 10 *c*), but often the flattened tip grows unequally and the unequally expanded tops on the slenderer lobes or stalk resemble splayed human feet (figs. *j–i* and 11 *b*). Also the gemmae show a tendency to form a separate thallus from each of the lobes at a growing point later presenting a turtle-like appearance (fig. 10 *a–e*).

TIBA, 1×10^{-3}M in White's solution. Gemmae treated with this solution, showed a somewhat retarded growth. While some showed splitting, others produced spherical buds at the growing point. A few were normal but showed one auricle of the thallus bigger than the other, similar in shape to that reported from naphthalene vapors. But with continued treatment with this chemical, normal growth was considerably affected. The short thalli became flat, succulent and irregular.

However, treatment with 1×10^{-4}M solution, appeared to induce the formation of a broad fan-like thallus from only one of the growing points, accompanied by splitting (fig. 13 *a, b*). The rim of the split was thick and dark green while the rest of the thallus appeared bleached.

Dowicide, 0.5 gm/liter, for 5 mts. and then on White's solution. Treatment with this herbicide even with very dilute solutions, proved highly lethal to the gemmae. The margin and the peripheral layers turned brown while the central portion and the apical notch alone remained alive. Injury with this chemical resulted in the formation of short cylindrical or spherical buds at the growing points (fig. 14 *a–c*). This produced short thickened thalli sometimes more than two, either from the upper or the lower surface of the gemmae that apparently did not show any injury, recalling growth on colchicine solutions (fig. 14 *d–j*).

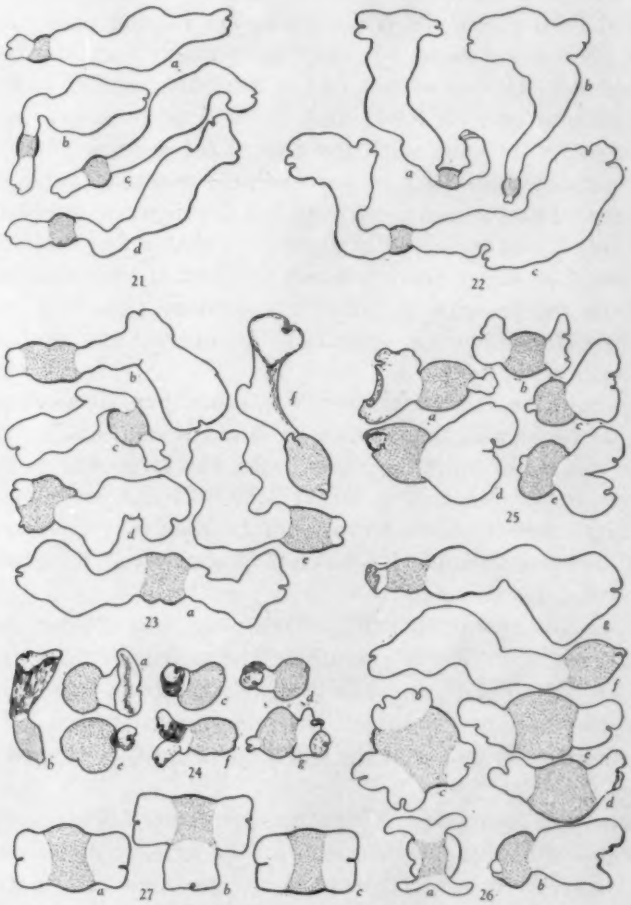
Acenaphthene. The dosage here is uncertain because of the volatility of the chemical. A small crystal was put in the petri dish with the gemmae on wet filter paper for 48 hours. A week after treatment, the gemmae elongated considerably. Two long pointed auricles were formed at each end of the gemma. Thus far, the development resembles that of gemmae treated

with 2,4-D, but the auricles of these plants never wrap around each other (fig. 15 *a, b*). In about another week dome-shaped buds arose between the auricles (fig. 15 *a, c*). Here again there is a difference from the gemmae treated with 2,4-D, for in this set, buds are never formed on the lobes but always between them whereas in the 2,4-D set, buds were formed almost indifferently, on or between the lobes. The dome-shaped buds soon grew into upright cylindrical structures (fig. 15 *d–h*) but never into normal flat thalli. After they had grown upright for a time, they began to spread at their upper ends and form flattened structures which were sometimes cuplike but more often one-sided in growth, so that they resembled a foot more closely than the similar structures which were mentioned in the discussion of the set treated with 2,4-D (fig. 15 *g–p*). Eventually, these flat upper extensions, which are remarkably large on some gemmae, (fig. 15 *q*) grow out into flat thalli. All these extensions, however, are found to grow out from only one of the growing points of a gemma.

Sodium cacodylate, concentration 1×10^{-2}M. Gemmae treated with sodium cacodylate expanded to approximately twice their original size. Figure 16 *a–m* shows representative shapes formed in the development from gemmae treated with this chemical. In most gemmae growth was retarded, although they were green and succulent. Pairs of short lobes appeared at the growing ends. Later, buds appeared between the lobes (fig. 16 *a, b, d, g*) and formed upright stalks but these were scarcely started before the upper ends began to flatten out and produce appendages, which were usually roughly crescent-shaped in outline (fig. 16, *e, h, i*). These were doubtless the equivalents of similar structures produced by treatment with 2,4-D and acenaphthene, but they differed in having very short stalks and in possessing markedly different shapes of terminal structures. Often they expanded into irregular thick thalli, which stood up vertically from the upper surfaces of the gemmae (fig. 16 *j, k, m*). It is remarkable that two unrelated chemicals such as acenaphthene and sodium cacodylate can produce structures so much alike in general as the upright structures shown in figures 15 and 16 and yet differ so much in ultimate detail as those figured in figure 15 *i–n*, from those shown in fig. 16 *e, h, i*. It must be emphasized that the shapes figured here were not variations selected to emphasize differences but were typical of the results produced by the different chemicals and were seen again and again. Perhaps we are laboring this point, but the shapes were so remarkable that it seems necessary to emphasize the fact that the drawings were all made from typical forms.

Actidione, concentration 0.01 gram per liter. Gemmae were treated with this antibiotic for 48 hours and then grown on White's solution. They showed comparatively little growth but usually formed an irregular lobe from each end. Later numerous thick, succulent lobes and buds were de-

veloped from the upper and lower surfaces of the gemmae (fig. 17 *b* and *c*). Small, wart-like knobs were also formed in great number from the upper and only from the upper surfaces of the gemmae (fig. 17 *c*). The number of knobs or spherical buds was small if normal thallus development occurred from the growing points (fig. 17 *a*). In other words, apical dominance decreased the number of adventitious buds on the surfaces of the gemmae.



Parathion, concentration 1 gram per liter. Gemmae were treated for 48 hours and then grown on White's solution. Apart from the splitting into an upper and a lower branch (fig. 5), which has been reported earlier in this paper, other shapes are produced. In early stages of growth the two auricles grew out from either end of each gemma and while one of these enlarged the other went on to a second dichotomy (fig. 18 *a* and *d*). Rhi-

zoids were usually abundant on the upper surfaces of the gemmae at this stage. The later growths were extremely irregular.

Naphthalene, dosage uncertain. Gemmae were exposed to the vapors of this substance. Their growth was slight and thallus formation was much delayed. The tips were developed with the growing points displaced to one side (fig. 19 *a–d*).

Maleic hydrazide, concentration 0.5% and 1.0%. Gemmae treated for 48 hours and then grown on White's solution formed patterns much like those given by acenaphthene, but they grew much more slowly. Two long pointed lobes were formed at each end of a gemma, similar to those treated with acenaphthene or with 2,4-D. But in these no buds ever appeared between the lobes, on the lobes or on any part of the gemmae (fig. 20 *a* and *b*). There was complete inhibition of any thallus formation. Some of the lobes became thickened and began to turn up but development proceeded no further. However, it was accidentally discovered that if the inhibited gemmae were perforated or sliced, the inhibition was lost at once and buds rapidly grew out from the growing points of the gemmae (fig. 20 *c* and *d*). This method of breaking inhibition appears to be unusual and we hope to make a further study of it.

Benzoguanimine, concentration 0.1 gram per liter. Gemmae were treated for 48 hours and then grown on White's solution. Enlargement of the gemmae was slight but a very small lobe was formed from one end and a larger one at the other. The larger lobe was characteristically rather narrow at first; then it widened considerably, then narrowed again (fig. 21 *a* and *b*). There was a tendency toward displacement of the growing points to the sides (fig. 2 *c* and *d*).

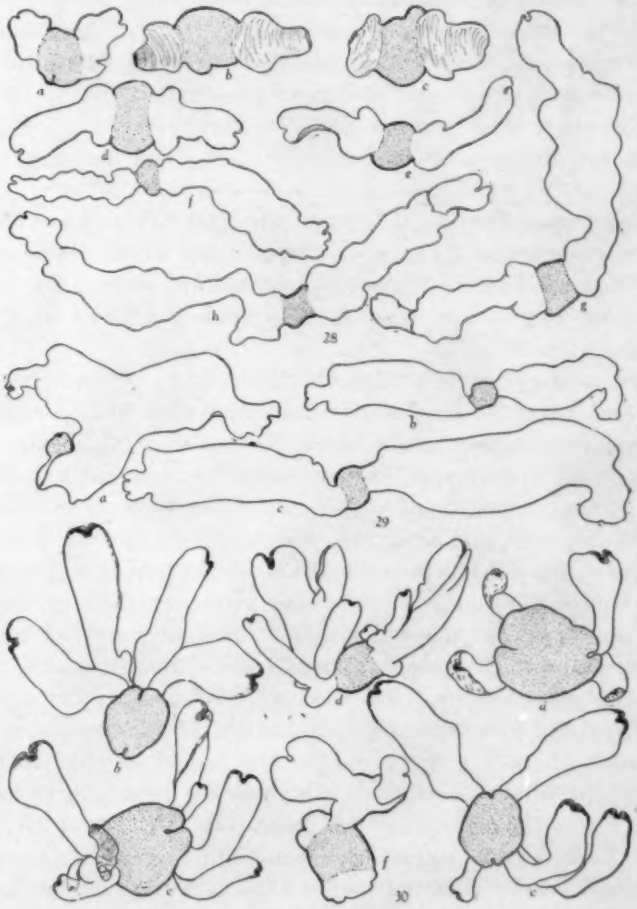
Formalin, concentration 0.01%. Treatment was applied for 48 hours followed by growth on White's solution. The gemmae thus treated showed very little growth, and thalli usually developed from one growing point only. These thallus lobes expanded irregularly and showed a tendency to develop fresh growing points at the sides. In this way T-shaped thalli were formed (fig. 22 *a–c*).

Coumarin, concentration 0.5 grams per liter. Lobes came out from gemmae treated with this chemical with a very wide, rounded sinus between them (fig. 23 *a–e*). No other treatment has given so wide a gap between lobes. Thalli grown for two weeks on White's solution plus coumarin tended to show wide, sweeping curves. Occasionally, rounded upright lobes were formed (fig. 23 *f*).

Ether—dosage uncertain. Gemmae treated with ether showed very limited growth. Commonly a succulent, cylindrical upright lobe developed from one growing point to a gemma and only an aborted one, or none, from the other growing point (fig. 24 *c* and *d*). Split tips also were induced (fig. 24

a and *d*). Later the cylindrical lobes unfolded as a rosette. In still others there was a definite tendency toward double dichotomy of the tips (fig. 25 *a–e*).

Phenol, dosage uncertain. Very dilute solutions were used. Some parts of the gemmae were killed. From some gemmae two lobes protruded from one end, became elongated and thickened with the tips bent back toward



the gemmae (fig. 26 *a*). In a few, the lobes fanned out in a rather extravagant fashion from gemmae which have grown little themselves and have formed only very small lobes from one end (fig. 26 *b*). In others extra growing points were induced (fig. 26 *c*), or the lobes of a growing thallus were unequal in size (cf. naphthalene), (fig. 26 *d* and *e*). Ultimately the thalli grew to be somewhat irregular in outline with a hump on one side (fig. 26 *f* and *g*).

zoids were usually abundant on the upper surfaces of the gemmae at this stage. The later growths were extremely irregular.

Naphthalene, dosage uncertain. Gemmae were exposed to the vapors of this substance. Their growth was slight and thallus formation was much delayed. The tips were developed with the growing points displaced to one side (fig. 19 *a–d*).

Maleic hydrazide, concentration 0.5% and 1.0%. Gemmae treated for 48 hours and then grown on White's solution formed patterns much like those given by acenaphthene, but they grew much more slowly. Two long pointed lobes were formed at each end of a gemma, similar to those treated with acenaphthene or with 2,4-D. But in these no buds ever appeared between the lobes, on the lobes or on any part of the gemmae (fig. 20 *a* and *b*). There was complete inhibition of any thallus formation. Some of the lobes became thickened and began to turn up but development proceeded no further. However, it was accidentally discovered that if the inhibited gemmae were perforated or sliced, the inhibition was lost at once and buds rapidly grew out from the growing points of the gemmae (fig. 20 *c* and *d*). This method of breaking inhibition appears to be unusual and we hope to make a further study of it.

Benzoguanimine, concentration 0.1 gram per liter. Gemmae were treated for 48 hours and then grown on White's solution. Enlargement of the gemmae was slight but a very small lobe was formed from one end and a larger one at the other. The larger lobe was characteristically rather narrow at first; then it widened considerably, then narrowed again (fig. 21 *a* and *b*). There was a tendency toward displacement of the growing points to the sides (fig. 2 *c* and *d*).

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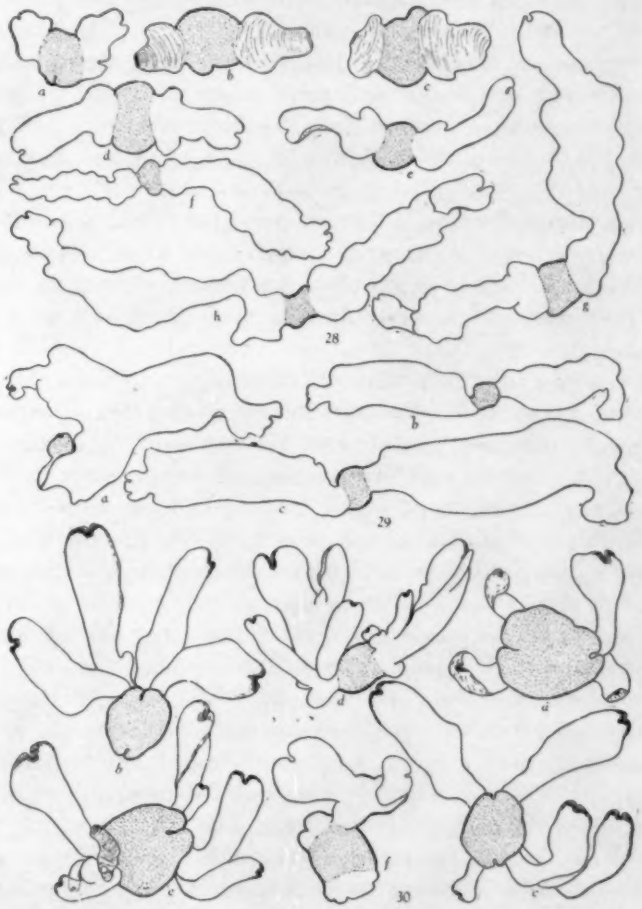


the gemmae (fig. 26 *a*). In a few, the lobes fanned out in a rather extravagant fashion from gemmae which have grown little themselves and have formed only very small lobes from one end (fig. 26 *b*). In others extra growing points were induced (fig. 26 *c*), or the lobes of a growing thallus were unequal in size (cf. naphthalene), (fig. 26 *d* and *e*). Ultimately the thalli grew to be somewhat irregular in outline with a hump on one side (fig. 26 *f* and *g*).

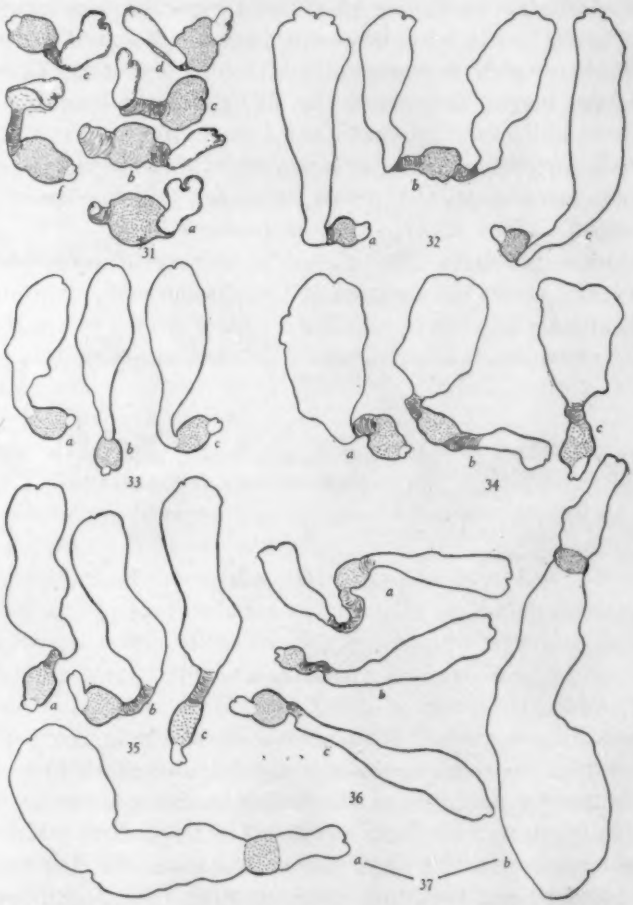
Diphenylamine. Of this substance a solution of 0.01 gram per liter (of the nutrient solution) was used. In early stages it induced growth from each growing point of the gemmae and a production of lobes with unusually square corners. The gemmae themselves grew little in length and not much in breadth. The appearance of the plants at this stage strongly resembled bow ties (fig. 27 *a–c*). In some examples a side branch was developed which also had very square corners and came out at precise right angles from the main body of the plant (fig. 27 *b*). The thalli soon lost their very regular appearance and developed a sort of segmentation. Later, these forms became still longer and more attenuated with sinuate margins replacing the strong segmentation. Shapes in the development of the "worm-like" thalli are represented in figure 28. The lobes are dark green and thickened.

Thiourea, concentration 0.1 gram per liter. This substance gave almost normal growth with long, strap-shaped lobes which developed rapidly. Suddenly the lobes became subject to very abrupt narrowing, after which new lobes grew out, which were scarcely more than half as wide as the original lobes (fig. 29).

The effect of growing plants on colchicine. Gemmae were treated with colchicine 1×10^{-2}M for 48 hours and were then put on equal parts of the colchicine solution and a solution of White's minerals on filter paper in petri dishes in the greenhouse. The gemmae were apparently dormant, dark green and somewhat succulent. After two weeks they were transferred to White's solution only, and after one month they were examined. Contrary to the results with brief treatment with colchicine, which causes much splitting of the tips of the thallus lobes as they grow out, these gemmae did not show splitting at all but showed flat, thickened lobes arising mainly from the lower surfaces of the gemmae (fig. 30 *a–c*), but occasionally from the upper surfaces or sometimes from both (fig. 30 *d* and *f*). The gemmae tend to remain flat disks with only slight expansion. The formation of numerous adventitious thalli occurs more toward the apical notches than between them, and surprisingly no rhizoids are observed to originate either from below or on top of the gemmae at this stage. The thick, succulent lobes bear some resemblance to those induced by acenaphthene but they are more variable. No rhizoids were observed to be formed from either surface at this stage.

The effect of various chemicals on the later growth of thalli. It has been reported above that klinostat experiments showed that *splitting* was independent of the pull of gravitation but always parallel to the flat surface of the splitting thallus. The klinostat tests were run in diffuse light, and as a result the thalli began to form long, slender tips, which turned upright and became cylindrical lobes which then flattened out on

top (fig. 31). Following this development, the thalli were removed from the klinostat and put on White's solution in petri dishes in the greenhouse. The thalli grew into flat plants, but retained distinguishable differences. The controls, which had been kept in the klinostat but were otherwise untreated, grew more rapidly than the others and developed a large lobe



from one end of each gemma and a small one from the other (fig. 37 *a* and). Almost none of the others developed more than hints of small lobes. The closest resemblance is seen between caffeine-treated and colchicine-treated gemmae. But it is readily seen that the gemmae treated with colchicine have formed lobes with broader ends (fig. 32) than those from caffeine-treated gemmae (fig. 36). What is more striking, though not well shown in the camera-lucida drawings, is that the lobes from the colchicine treated plants are upright while those of the caffeine-treated set lie prone.

There is a basic resemblance between the plants from acenaphthene-treated gemmae and naphthalene-treated ones, but those produced by naphthalene have lobes which widen out and then round off gently to the tip (fig. 33) while those resulting from acenaphthene widen, then are constricted, then widen again (fig. 35).

Sodium cacodylate treatment produces the most characteristic form of the group (fig. 34). The lobes grow out from the gemmae as very narrow necks. Then they widen very abruptly and form spatulate lobes nearly as wide as they are long. The controls (fig. 37) appear to be normal and more vigorous.

It is rather surprising that after a growth of seven weeks the distinctions due to different treatments still persist, although they are less striking than the earlier ones.

Chromosome numbers. The use of a number of polyploidizing substances naturally raises the question of the chromosome condition or condi-

TABLE 1. *Chromosome numbers of plants of Lunularia grown from gemmae treated with various chemicals.*

Chemicals used in treatment	Concentration of chemical used	Number of figures showing 7 chromosomes	Number of figures showing 14 chromosomes	Number of figures showing other than 7 or 14 chromosomes
acenaphthene	small crystal to one petri dish	4	1	0
colchicine	1×10^{-2} molar	5	1	0
sodium cacodylate	1×10^{-2} molar	0	1	0
ether	uncertain	6	0	0
2,4-D	0.1%	8	0	0

tions of these bizarre forms. Chromosome studies have not yet been completed, but a few observations make it seem doubtful whether the strange forms are caused by polyploidy. The basic chromosome number for *Lunularia* has been given as 8 by Heitz (1927). The few counts which have been made did not agree with the observations of Heitz. We found the haploid number to be seven and found no numbers other than 7 or 14. Our counts are given in Table 1.

While diploids are seen to be common, the commonest number of chromosomes is 7. As has been mentioned, the rather remarkable uniformity of the plants resulting from any given treatment leads us to think that, although there may have been diploids among these plants, and there almost certainly were, they did not show morphological features distinguishable from those of the haploids. No segregation of different types has been possible in any set of plants resulting from any one of our treatments. It is our intention to pursue this phase of our studies far enough in the future to give

assurance of our knowledge of the past played by diploidy and polyploidy in the morphological development of these types.

Ultimate fate of the chemically induced morphological variants. Sad indeed would be the fate of the bryologist confronted with the necessity of taxonomic treatment of such a varied set of forms as we presented in this paper. Comfort can be given to the taxonomists by the statement that, so far as we have been able to determine, no one of these variations persists for any considerable length of time and none is able to reproduce itself. Whatever the variations through which a plant passes: spreading lobes which split; upright cylindrical lobes which flatten on top producing what we have facetiously called "feet"; etc., it is destined to produce a more or less normal thallus. As the gemmae from which they came die off and the basal parts of the thalli die and decay, no trace will be found in any of them of a form so different from this final one as those we have attempted to depict.

Discussion. We have tried to make some sort of classification of this rather bewildering assemblage of forms in the hope of arriving not at an explanation of these manifestations, but possibly of finding a correlation between certain growth forms and definite chemicals.

Normally a gemma, which has been removed from the gemmae cup which bore it and placed on a moist substratum, will respond by producing a fan-shaped thallus from each of its growing points. Usually a gemma has two growing points, but gemmae with three growing points are common. Those with more than three growing points are very rare.

Each fan-shaped thallus has a notch at its tip which contains an apical cell by which elongation of the thallus is produced. The gemma itself grows comparatively little. Gemmae treated with benzoguanimine, formalin, ether, naphthalene, colchicine, caffeine and sodium cacodylate were close to normal in this aspect. Gemmae treated with acenaphthene, 2,4-D, and maleic hydrazide often showed considerable enlargement.

Inhibition of one growing point so that growth took place only from the other was often produced by treatment with formalin, phenol, coumarin, naphthalene, sodium cacodylate, caffeine, and TIBA.

The splitting of the tips of the thalli produced from the growing points has been caused by acenaphthene, actidione, caffeine, chromic acid, ether, colchicine, naphthalene, parathion and sodium cacodylate.

Notable departures from the normal fan-shaped thallus which arises from a normal growing point were produced by treatment with formalin, ether, benzoguanimine, coumarin, phenol, diphenylamine, acenaphthene, naphthalene, sodium cacodylate, colchicine, caffeine, and 2,4-D. The outlines of these forms vary considerably but each is characteristic of a definite chemical. The margins are usually sinuous but in some examples of treatment with 2,4-D, they are irregularly toothed.

In some of the thalli from treated gemmae, the growing point was displaced to one side. This result was produced by coumarin, benzoguanimine, phenol, formalin, and ether. Sometimes, thallus tips were formed with notches on either side of the tips so placed as to form T-shaped structures. This was noted especially in examples produced by treatment with formalin and diphenylamine.

Thalli which appeared to be segmented were produced by 2,4-D, and diphenylamine. Diphenylamine produced thalli which had a worm-like appearance but 2,4-D gave much more irregular thalli with joints suggestive of those of arthropods.

It has been noted that gemmae treated with 2,4-D, maleic hydrazide and acenaphthene grow considerably and become succulent although the apical cells are more or less completely suppressed. Such gemmae form long pointed lobes on either side of each apical cell. These points were rather short in gemmae treated with acenaphthene, but long in those treated with maleic hydrazide and 2,4-D. In the 2,4-D-treated gemmae the points may be widely spread, even reflexed, or wrapped around each other.

Another strange type of development was that of upright, cylindrical lobes, induced by several substances. Acenaphthene caused the growth of initials between the long points previously mentioned. After growing upright for a time these lobes become flattened on top and produce structures resembling human feet. Sodium cacodylate and ether stimulated the formation of similar structures which, however, possessed much shorter upright portions and flattened tops, usually crescent-shaped in outline. 2,4-D caused the appearance of many irregular, upright lobes which sometimes arose from initials between the points but often also from buds on the points or even on the bodies of the gemmae.

Buds, not from growing points, but elsewhere on the gemmae were induced by colchicine, sodium cacodylate and 2,4-D. Actidione caused the formation of great numbers of such buds.

In spite of the preceding groupings it is impossible to deny that great differences exist between the subjects of the various treatments, and that any one set is distinguishable from any other. At present we can only note the differences without being able to explain them. It is not possible to group the chemicals in any way to correlate them effectively with the groups of forms we have just arranged. Some of the chemicals are acids, some basic, some are volatile, some readily soluble in water, some soluble only with difficulty, some are organic, others inorganic. Altogether, we have employed a most miscellaneous group of chemicals and we can do little more than report their effects.

The splitting of the tips of the young thalli was produced by a number of substances. Some of these caused the abnormal type of branching in a

high percentage of treated gemmae. Others produced it more sparsely, or even rarely; some not at all. Though it has been observed in controls, i.e., gemmae grown in petri dishes on filter paper with White's solution, it has never been found in *Lunularia* or *Marchantia* in the greenhouse, or on *Marchantia* in the field. Leaves with enations parallel to the surface of the leaf blades have been produced in a fern by the application of auxin by Labouriau (1952). Halbsguth (1936) has shown that splitting is produced by the formation of two apical cells, one above the other. From the present study it appears to us that the formation of two such apical cells may occur in any young thallus tip which has been caused to thicken by any means. Thus any factor which could temporarily inhibit the elongation of the young thallus tip might cause a thickening of that tip and consequently set up the mechanism of splitting. This hypothesis would reduce the effects of the various factors which cause splitting to a simple inhibition, and may explain how splitting can be induced by so many apparently unrelated substances. From the drawings we have presented it is obvious that, if growth takes place from even one initial of a gemma, the enlargement of the gemma itself is thereby inhibited. But if the initials be inhibited in growth, the gemmae may grow considerably as shown by the sets treated with actidione, maleic hydrazide, and 2,4-D. These are simple cases of inhibition of growing points.

Apparently the inhibition of growing points for a time, with a subsequent loss of inhibition, gives rise to cylindrical, upright lobes such as are illustrated for the result of the treatment with actidione. Why the delayed development of the initials should cause them to grow into cylindrical structures rather than flat thalli, or why the cylindrical lobes grow upright instead of lying flat, we cannot guess.

The upper ends of the cylindrical, upright lobes tend to develop into expanded thalli which at an early stage curiously resemble human feet in shape. Similar flattenings on the lobes caused by sodium cacodylate and ether are inclined to form crescent-shaped flat ends. Cylindrical lobes due to 2,4-D treatment usually form more irregular cups. If these cups represent a natural tendency of the lobes to broaden out and form normal thalli, why should different chemicals produce cylindrical lobes of differing lengths and cups of differing shapes?

The inhibition of growing points with 2,4-D, sodium cacodylate, and especially by actidione seems to serve as a removal of the dominance of these points and to allow the formation of buds on thallus points or on the bodies of the thalli. But why does actidione produce so many more buds than the others?

We have seen no evidence of stimulation by any of these chemicals, and we admit that all the variations in morphology we have described may be due to injury or injuries of one kind or another. But we cannot see how in-

jury induces *splitting*, nor can we avoid the assumption that there must be injuries of many types to produce such a variety of forms from a very uniform lot of gemmae.

SUMMARY

1. Gemmae of *Lunularia* were treated with a miscellaneous assortment of chemicals which bore no particular relation to one another.

2. The outgrowths from treated gemmae showed strange and unusual morphological divergences from normal development.

3. Each chemical produced a characteristic morphological type, different from all others.

4. Although a rough grouping could be made of certain growth reactions, each chemical caused minor variations which elude classification.

5. An attempt has been made to explain the abnormal morphological variations on the general basis of injury, inhibition, and removal of apical dominance.

6. These bases are unable to explain the large number of minor variations which were so striking a feature of the responses induced by these chemicals.

7. No permanent effects were provided by the chemical treatments here described.

8. We do not believe that the observed morphological changes can be explained by chromosomal changes.

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PROSORI IN SYNCHYTRIUM[1]

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In some species of *Synchytrium* the initial cell or thallus derived from a zoospore infection functions as a prosorus and gives rise to a sorus of sporangia within the infected cell. In this process the content of the mature initial thallus grows or flows out slowly through a pore in its wall to form an attached vesicle of protoplasm which becomes enveloped by a wall of its own, undergoes cleavage into sporangia, and is thusly transformed into a sorus. Usually as it becomes empty and the incipient sorus expands against it, the prosorus is invaginated and later appears as a collapsed vesicle below, above, or at the side of the sorus. Oftentimes it is so compressed and distorted within the infected cell that its presence may be overlooked. Nevertheless, it is usually attached to the sorus in the region of the pore through which its protoplasm formerly flowed, and the place of attachment is clearly evident as a thickening in the wall or a plug of densely stainable material as Rytz (1907) and the author (1954b, 1955c) have shown in several species. In living as well as in fixed and stained sections this plug is clearly recognizable as an opaque, refractive, or deeply-stainable area, and its presence indicates the occurrence of a prosorus although the latter may be hardly recognizable as such in the collapsed condition.

The resting spore or zygote also may function as a prosorus during germination in some species of *Synchytrium* while in others it may be transformed into one sporangium, or a sorus of sporangia. The occurrence of prosori in the life cycle of a species is a sharply-defined criterion or classification, and most students of *Synchytrium* recognize it as a clear-cut subgeneric distinction. According to the most recent keys to the subgenera, all species in which the initial cell and resting spore function as a prosorus belong in *Microsynchytrium*, and similar species in which the resting spore is transformed directly into a sporangium during germination belong in *Mesochytrium*. Therefore, it is essential to know whether prosori are present before a species can be classified correctly.

Until recently the presence of prosori was known in relatively few species, but intensive study of old and new species by the author and his students during the past decade have shown that their occurrence is fairly common in the genus. The author (1954b & c; 1955a, b & c) discovered their presence in *S. australe, S. brownii, S. ranunculi, S. lindquistii, S. achryo-*

[1] This study has been supported by a grant from the National Science Foundation.
[2] The author is very grateful to the late Dr. M. T. Cook for prepared slides and herbarium material which he generously contributed shortly before his death.

clines, S. chiltoni, S. cerastii, and *Synchytrium* sp., species in which they had been overlooked. Lingappa (1953, 1955) also has found that the initial cell of *S. collapsum, S. lepidagathidis, S. cookii, S. oroxyli, S. crustaceum, S. zorniae, S. cassiae, S. maculans, S. trichodesmatis, S. biophyti, S. oldenlandiae, S. launeae, S. phyllanthi, S. sesamicola* and *S. akshaiberi* functions as prosorus. More recently the author has found that a prosorus is developed from the initial cell of additional species in which it was previously unknown. The present contribution concerns its occurrence in these species as well as a description of other structural characteristics of such species and host reactions which are significant to their classification.

Synchytrium perforatum. This species was collected first by W. N. Suksdorf on *Valeriana sitchensis* in 1885 and 1886 on Mt. Adams, Washington, and later (1908) on *Valerianella congesta* in Hood River County, Oregon, according to Cook and Shaw (1953) and herbarium material deposited at the State College of Washington. These herbarium specimens, kindly loaned to the author by Dr. C. G. Shaw, bear the name *Synchytrium perforatum* Ellis, which indicates that Suksdorf sent them to Ellis for identification. Ellis, and Cooke and Shaw did not describe this species, and Cook (1951) and Cash (1954) merely mentioned it by name. The same species was collected by the author in 1954 on *Valerianella stenocarpa* at College Station, Texas and transferred with its host to Purdue University where both flourished fairly well. The present contribution is based on a study of this material and a comparison with the herbarium specimens from Washington and Oregon.

The initial cell or thallus of *S. perforatum* functions as a prosorus and gives rise to a sorus of sporangia as shown in figures 1 and 2. In these figures the sori lie below the empty and collapsed prosori, and both are enveloped by a fair amount of host protoplasmic residue (fig. 1). Particularly conspicuous is a thickened area or plug of material in the region formerly occupied by the exit pore between the walls of the prosorus and sorus (figs. 1–3). This area or plug is fundamentally similar to that shown by the author (1955a) in *S. australe,* and its presence, as will be shown in other species also, is a clear indication that the initial cell has functioned as a prosorus. So far empty prosori have been found only in the apex of the host cell, but it is not improbable that they may occur occasionally at the side or below the sorus. At present *S. perforatum* appears to be similar to *S. stellariae* and *S. cerastii* with respect to the relative position of the empty prosori.

Synchytrium perforatum stimulates the development of sporangial and resting spore galls on its hosts which are fundamentally similar in being composite and multicellular. These galls may be single and scattered on the host, but they are commonly aggregated and frequently confluent. In

the latter instances the infected portions of the leaves may become 3 to 4 times their normal diameter. Both types of galls are embedded largely in the host tissue, and protrude only slightly above the surface. However, considerable proliferation of the host tissue occurs around the sites of infection so that raised humps of tissue are formed in which the galls occur. The sporangial galls are often narrowly to broad pyriform in shape, 180–240 × 204–360 μ, with a sheath 2–4 cells in thickness around the infected cell (figs. 1 & 2). The outermost cells of the sheath contain a small amount of anthocyanin which imparts a slightly lavender-red color to the galls. The resting spore galls are usually larger than the sporangial galls, oval to pyriform in shape, and vary from 290–375 μ high by 400–480 μ broad (fig. 4).

The successive developmental stages of the galls have not been studied intensively, but present evidence indicates that they are formed by enlargement of the infected epidermal cell and division of the adjacent and bordering cells. So far division of the infected cell as in *S. australe* has not been observed. The sheath cells surrounding the infected cell are comparatively small, thin-walled and meristematic with conspicuous nuclei and fairly dense cytoplasm, and in median cross sections of mature galls they are somewhat radially oriented (fig. 3). A few of them have been found dividing even after the prosorus is empty (figs. 1 & 3). That the adjacent epidermal cells may divide also is indicated by the cell at A in figure 1. Accordingly, infection by *S. perforatum* apparently results in enlargement of the infected cell and division of the adjacent healthy cells.

However, the most conspicuous reaction of the host involves the wall of the infected cell. Usually, it becomes greatly thickened, lignified and perforated. In some sporangial galls it may not be greatly thickened or perforated (fig. 1), but in others (figs. 2 & 3) the thickening and perforations are quite conspicuous. In resting spore galls particularly the wall varies from 5–17 μ in thickness and may be perforated by 28–90 oval, 10–18 × 20–32 μ, almost circular, 8–18 μ, oblong, 10–16 × 17–35 μ, elongate to broadly or narrowly fusiform openings (figs. 5 to 7). These may be surrounded by an optically differentiated zone, 7–14 μ broad (fig. 8). The thickening and lignification of the host cell wall make the gall hard and tough in texture, and they frequently feel like grains of sand. As the host tissue disintegrates, the thin-walled sheath gradually sloughs off (fig. 7), leaving the infected cell and its resting spore intact. Such cells have the appearance of minute, narrowly to broadly pyriform perforated vessels with a small globule (resting spore) in the base (figs. 5 to 7).

The reaction noted above has been found in all reported hosts of this species, including *Valeriana sitchensis, Valerianella congesta* and *V. stenocarpa,* and at present it appears to be a characteristic response to infection by *S. perforatum*. The name given to this species by Ellis is very descrip-

tive of the outstanding host reaction and should be retained, although he did not describe or diagnose the fungus.

Other striking cellular reactions found in *Valerianella stenocarpa* involve enlargement and distortion of the host nucleus and an accumulation of cytoplasm in the host cell. As the infected cell enlarges its nucleus increases in size also so that by the time the resting spores reach definitive size it may be as much as 50 μ in diameter. Furthermore, it becomes irregular in shape and deeply-lobed (fig. 4) like the nuclei of host cells infected by *S. anemones, S. mercurialis, S. anomalum, S. chrysosplenii,* etc. Numerous densely chromatic bodies may be present in its interior which look like fragments of the nucleolus. The nucleus persists for a long time and apparently does not begin to disintegrate until after the resting spores are mature. At least this is indicated in figure 4 of a gall with a mature resting spore.

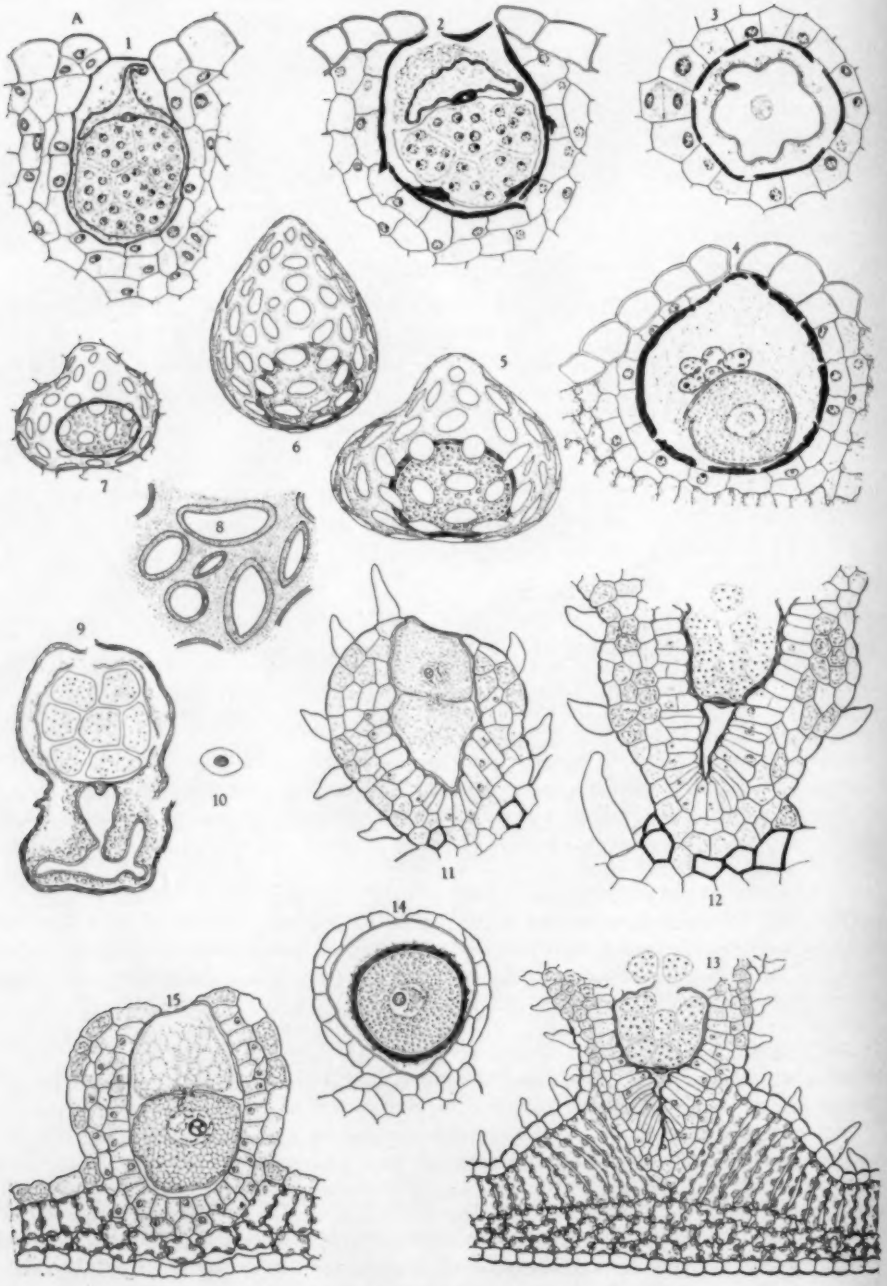
A marked increase in cytoplasm also occurs in the infected cells. It is more abundant in cells which bear the prosorus and sorus, and this is reflected later in the greater amount of residue around these structures (figs. 1 & 2). In most cells bearing resting spores the cytoplasm is quite abundant also and appears reticulate and foamy (fig. 4), but comparatively few globules and granules are present in it. Accordingly, very little if any residue is present around most of the mature resting spores of *S. perforatum* on *V. stenocarpa.*

Inasmuch as there is no description of *S. perforatum* in the literature, the following diagnosis is presented herewith:

Prosori usually solitary, occasionally 2 in a cell, subspherical, 98–130 μ, or oval 95–108 × 110–135 μ, partly filling host cell; lying in apex of cell when empty. Sori subspherical, 115–144 μ, oval 85–110 × 98–148 μ, with dark-orange granular content and a relatively thin hyaline wall; usually lying beneath empty prosorus. Sporangia 12–22 per sorus, predominantly polyhedral, 24–48 μ in greatest diam., with dark-orange content and a thin hyaline wall. Zoospores subspherical to oval after emerging, becoming more elongate and almost oblong while actively motile, 2.9–3.1 × 3.3–4 μ, with a refringent orange globule. Resting spores usually solitary and filling only a part of the host cell, occasionally 2–3 in cell, spherical, 38–72 μ, oval, 52–65 × 70–78 μ, with reddish-brown coarsely granular content and a smooth 3–4 μ thick wall, surrounded occasionally by a thin layer of residue; germination unknown.

Sporangial and resting spore galls composite and multicellular, single and scattered, but commonly aggregated and frequently confluent, usually embedded largely in the host tissue. Sporangial galls usually broadly to narrowly pyriform, 180–240 × 204–360 μ, with a sheath 2–4 cells thick. Resting spore galls oval to pyriform, 290–375 μ high by 400–480 μ broad.

On the leaves, petioles and stems of *Valeriana sitchensis* in Washington, *Valerianella congesta* in Oregon (Suksdorf) and *V. stenocarpa* in Texas (Karling), causing thickening and distortion of the infected organs.



FIGS. 1–8. *Synchytrium perforatum*. FIGS. 9 & 10. *S. valerianellae*. FIGS. 11–14. *S. holwayi*. FIG. 15. *S. chamaedryoides*. FIGS. 1 & 2. Longitudinal section of em-

Synchytrium valerianellae. Inasmuch as this species occurs on a host closely related to those of *S. perforatum*, the author was anxious to determine whether or not the two parasites are identical or closely related. *Synchytrium valerianellae* was collected on *Valerianella truncata* in Greece and described by Sydow (1935), who apparently believed that the sorus develops directly from the initial cell or thallus. He, accordingly, placed it in the subgenus *Eusynchytrium*. Type material of Sydow's species was secured from the Naturhistoriska Riksmuseet, Stockholm, Sweden, through the courtesy of Dr. Sten Ahlner, for comparison with *S. perforatum*. Small pieces of infected leaves were soaked thoroughly in water, placed in fixatives in a vacuum to remove air, embedded, sectioned and stained. Although sections of such material were shriveled and shrunken the sporangial galls were sufficiently well preserved to show that the initial cell functions as a prosorus and gives rise to a sorus of sporangia as in *S. perforatum*. No migration stages of the protoplast in the development of the sorus were observed, but several empty and collapsed prosori were found in the base of infected cells. Also, the characteristic thickening of the wall or plug was present in the region of the former connecting pore in all cases, which is additional evidence for the presence of a prosorus in this species. This is illustrated in figure 9 of a badly distorted and shrunken infected cell with a sorus of sporangia above the empty prosorus. The shrunken sheath cells around the infected one are omitted in this drawing. In this figure the so-called plug projects downward into the empty prosorus as a conical protuberance, and may be almost circular to oval, 5–7 × 6–8.4 μ in diameter, at the base (fig. 10). So far only 22 empty prosori have been observed, and all of these occurred in the base of the cell. Although no early stages of sorus development were found in Sydow's material, the author is convinced from the mature stages observed that the presence of a prosorus will be confirmed when living and more favorable material is studied.

Sydow made no distinctions between sporangial and resting spore galls, but sections of his material showed that they are basically similar in being composite and multicellular. Also, the incipient sheath cells apparently become infected so that both types of galls frequently become compound. As

bedded sporangial galls with empty prosori in apex of infected cell. × 140. FIG. 3. Cross section of sporangial gall showing prosorus and plug in median view. × 140. FIG. 4. Longitudinal section of resting spore gall showing thickened and perforated wall of infected cell, resting spore, and lobed host nucleus. × 140. FIGS. 5–7. Pyriform infected cells without sheaths showing perforations in the walls. × 188. FIG. 8. Variations in size and shape of perforations or openings. × 360. FIG. 9, Infected cell with empty prosorus below sorus of sporangia. × 210. FIG. 10. Plug in cross section. × 540. FIG. 11. Sporangial gall on stem with protoplasm migrating into incipient sorus. × 200. FIG. 12. Cupulate sporangial gall; empty prosorus being invaginated by elongating sheath cells in base. × 233. FIG. 13. Later stage in same process; palisade parenchyma cell greatly elongate. × 150. FIG. 14. Isolated resting spore gall on stem. × 188. FIG. 15. Isolated sporangial gall on leaf with protoplasm migrating into incipient basal sorus. × 288.

many as 5 infected sheath cells of a gall have been found. Both types of galls may be single and scattered or aggregated and confluent, and protrude prominently on both surfaces of the leaves. The sporangial galls are usually broadly pyriform, 90–185 × 170–200 μ, to subspherical, 85–180 μ. The resting spore galls are fairly similar in shape, broadly pyriform, 120–216 μ × 160–240 to subspherical, 120–210 μ. The compound galls of both types are much larger in size and irregular in shape. The resting spores are enveloped by a large amount of residue which may sometimes fill the remainder of the cell.

Synchytrium valerianellae is similar to *S. perforatum* in being long-cycled, by inducing composite, multicellular sporangial and resting spore galls on its hosts, by developing a prosorus, and by its ability to infect species of *Valerianella*. Furthermore, the size and shape of its sori and sporangia as well as the number of sporangia per sorus fall within the range of those of *S. perforatum*. The chief known differences exhibited by *S. valerianellae* are the basal position of the empty prosorus in the infected cell, larger resting spores with a great amount of enveloping residue, and lack of induced perforations in the wall of the infected cell. The walls of cells infected by *S. valerianellae* may become 3–4.8 μ thick but they do not appear to be lignified, and in no instances were perforations observed. How significant this host reaction may be taxonomically is not certain at present. Possibly, *S. valerianellae* might induce the same reaction if it can be grown on *Valeriana sitchensis, Valerianella congesta* and *V. stenocarpa*, the hosts of *S. perforatum*. Such cross inoculations as well as host range and resting spore germination studies must be made before the identity of the two species can be settled. However, for the time being, at least, the author regards them as distinct.

Synchytrium holwayi. This species was collected first by Holway on *Monardia fistulosa* in Iowa and sent to Farlow (1885) who named it *S. holwayi*. Subsequently, it was reported on the same host by Greene (1946) in Wisconsin. Farlow was uncertain about the presence of a prosorus, although his figure 12 shows a collapsed vesicle in the apex of the infected cell which looks like an empty prosorus. In living and fixed, sectioned and stained material which Dr. H. C. Greene generously contributed from Wisconsin the author found evidence of a prosorus in all sporangial galls and thus confirmed Farlow's intimation that it might be present in this species.

Synchytrium holwayi induces the development of composite, multi-cellular sporangial and resting spore galls which usually protrude fairly prominently on the surface of the host. Occasionally, however, they may be immersed fairly deeply in the host tissue. Both types of galls may be single and scattered, but they are frequently aggregated and confluent, lavender- to purplish-red in color and usually papillose. The sporangial galls are

usually subspherical to broadly pyriform, 168–194 μ high by 82–252 μ broad, and become cupulate after dehiscing (figs. 12 & 13). The resting spore galls are usually smaller, subspherical, oval, or broadly pyriform, and 96–192 μ high by 72–228 μ broad (fig. 14).

As the sporangial galls develop the incipient sheath cells frequently become infected with the result that compound galls are formed as in *S. valerianellae*. Such galls may become unusually large and irregular in shape with the initially infected cell and its parasite in the center and 1–6 infected sheath cells at the periphery. The sheath surrounding the infected cell in sporangial galls may be 3–5 cells in thickness (figs. 11 & 12), whereas in resting spore galls it is relatively thin and usually consists of 1–2 cells (fig. 14). Many or most of the outer sheath cells are filled with anthocyanin which give the galls their characteristic color. In fixed and stained sections the content of such cells has the appearance of a coagulated resinogenous substance (figs. 11 to 13) very similar to that described by the author (1954b) for *S. australe*. In both types of galls the pore or opening to the infected cell may be narrow (fig. 14) or quite broad, so that in the latter instances a considerable part of the infected cell is exposed (fig. 11). Many of the outer cells of the sheath bear short swollen trichomes which give the galls a characteristic appearance. The presence and development of such hairs, however, is not a reaction of the host to infection because they are commonly abundant on healthy leaves and cells of *Monardia fistulosa*.

The presence and development of the sorus from a prosorus in *S. kolwayi* is clearly evident in figures 11 to 13. Figure 11 shows a migration stage of the protoplasm into the developing incipient sorus in the apex of the infected cell, and figures 12 and 13 show the empty collapsed prosorus under the sorus of sporangia. Farlow's figures of this species indicate that the empty prosorus lies in the apex of the infected cell, but in the Wisconsin material it occurred nearly always in the base. So far only two cases of its presence in the apex of the infected cell have been found.

In the dehiscence of the sporangial galls the sheath cells immediatly adjacent to the lower part of the host cell appear to play an important role. This consists of their elongation inward and upward whereby the infected cell and empty prosorus are compressed. This elongation may be noted fairly early and may become evident before the prosorus is completely empty, as shown in figure 11. It continues after the prosorus is empty and the sorus has undergone cleavage into sporangia (fig. 12). At the same time these cells may divide longitudinally and transversely, and those around the upper half of the host cell and sorus begin to elongate inward also (figs. 12 & 13). The result of this elongation and division of the inner sheath cells is an almost complete obliteration of the lower part of the infected cell and empty prosorus, so that these appear as a narrow irregular

cavity (fig. 13). Further elongation and division may fill up the lower part of the gall with cells, and this together with the growth of the cells around the upper half of the host cell probably exerts pressure on the sorus, causing it to rupture. While these internal changes are occurring the sheath cells at the apex of the gall begin to break loose, and probably as pressure is exerted from within they are pushed off and disappear. This exposes the sorus and sporangia, and after the latter have disappeared the empty gall becomes somewhat cupulate. The growth and elongation of the inner sheath cells and the part they seem to play in the dehiscence of the galls of *S. holwayi* appears to be fundamentally similar to those illustrated by Kusano (1907, fig. 8) for *S. minutum*.

Another host reaction to be noted here occurs in relation to isolated galls on the upper surface of leaves. This often involves the elongation of the palisade cells around the base of the galls. The palisade cells of *Monardia fistulosa* are normally up to 70 μ long, but those around the base of a gall may attain a length of 110–150 μ in some instances (fig. 13). As a result the base of the gall sometimes appears to sit in a raised cushion of palisade parenchyma.

Synchytrium chamaedryoides. This species was described by Cook (1952) as *S. urticae* on *Urtica chamaedryoides* in Louisiana. The author (1953a) pointed out that Cook's binomial is invalid because it had been used earlier by Sorokin (1873) for certain bodies which he found in the epidermal cells of *Urtica dioica* in Russia, and believed to be the resting spores of a *Synchytrium* species. A study of Sorokin's material as well as living leaves of *U. dioica* and *U. chamaedryoides* by the author (1953b) showed that these bodies do not relate to *Synchytrium* but are the stalked cystoliths which occur commonly in epidermal cells of the Urticaceae. In the same year the author collected Cook's fungus on *U. chamaedryoides* in Texas and Louisiana, verified it as a species of *Synchytrium*, and changed its name to *S. chamaedryoides*.

Cook described the sporangial galls, sori, and sporangia very briefly, but he failed to observe resting spores and the occurrence of a prosorus in the life cycle of this species. In living as well as in fixed, sectioned and stained material collected in Texas and Louisiana in 1953 and 1954 the author found that the initial cell is a prosorus which at maturity evacuates its content to form a sorus of sporangia. A late stage in this process is shown in figure 15 in which most of the protoplasm has passed into the incipient sorus in the base of the infected cell. Figures 16 and 17 show much later stages in which the sporangia are fully formed. In the majority of sporangial galls examined the empty, collapsed prosorus was lying in the apex of the infected cell, but this is not a general occurrence. It has been found at the side (fig. 16) and in the base (fig. 17) of the host cell also, so that its

position in relation to the sorus varies. Regardless of its position, its point of attachment to the sorus is clearly visible as a plug or thickening of the cell walls (figs. 16 & 17).

Synchytrium chamaedryoides induces the development of sporangial and resting spore galls on its hosts, and both of these are composite and multicellular and usually reddish-lavender in color at maturity. Occasionally, the incipient sheath cells become infected with the result that compound galls are formed. The galls may vary considerably in size and shape, depending on whether they occur singly and are isolated, or are aggregated and confluent on the leaves, petioles and stems. Isolated sporangial galls on the leaves usually protrude prominently, and frequently have the structure, shape and appearance shown in figure 15. The protruding portion may be somewhat broadly dome-shaped or nearly hemispherical and broadly crateriform, while the basal portion is embedded in the palisade or mesophyll tissues. The sheath may be 2 to 4 cells in thickness, and its cells may not be markedly enlarged. Those adjacent to the infected cell often have a radiate arrangement and may be elongated at right angles to the long axis of the host cell (fig. 15). In fixed and stained sections the outermost cells are usually filled with a coagulated shrunken substance which is probably due in part to the presence of anthocyanin in these cells. In some isolated sporangial galls on leaves the sheath was composed largely of greatly enlarged and outwardly elongated epidermal cells and their derivatives. Some of the epidermal cells were 144 μ long and wedge-shaped while others were divided transversely. It is, thus, apparent that the enlargment of the healthy sheath cells may vary markedly in the isolated galls on leaves. On the stem and petioles the sporangial galls may be closely crowded, and these are usually more elongate outwardly (fig. 16) than those on the leaves. Frequently, they are more deeply embedded in the host tissue. In the gall shown in figure 16 the sheath is two cells in thickness, and the outer cells are greatly enlarged. The inner cells are somewhat radially oriented on the infected cell and elongate. This is a rather common occurrence in galls on the stems. Figure 17 shows an isolated sporangial gall on the stem in which the sheath is 2 cells in thickness, but all of these cells are markedly enlarged. Some of them are 3–5 times larger than those shown in figure 15. The isolated sporangial galls on leaves vary from 150–192 μ high by 220–280 μ broad.

As noted previously the resting spore galls are similar in structure, but their sheath cells may not vary as greatly in size as those of the sporangial galls. Figure 18 shows an isolated gall in longitudinal section from Cook's slides, and in this figure, the protoplasmic residue around the resting spore almost fills the remainder of the host cell. Such galls vary from 117–156 μ high by 200–258 μ broad. Cook's measurements of the galls, sori and sporan-

gia (48×48—93–$156\ \mu$, $39 \times 39\ \mu$, and 8–$15\ \mu$, respectively) are incorrect. In his fixed and stained sections as well as in living material collected by the author the prosorus is almost spherical, 80–$112\ \mu$, or oval, 66–78×84–$98\ \mu$, with a 2–$2.8\ \mu$ thick wall; the sorus is subspherical, 76–$127\ \mu$, or oval, 54–80×75–$120\ \mu$; the zoospores are oval to oblong, 3.2–3.8×4–$4.6\ \mu$, when actively motile, and the resting spores are almost spherical, 60–$90\ \mu$, or oval, 58–60×70–$90\ \mu$, with a 2.8–$3.6\ \mu$ thick, smooth, amber to brown wall and coarsely granular content. Germination of the latter has not yet been observed. From these observations it is obvious that *S. chamaedryoides* is a long-cycled species which obviously does not belong to the subgenus *Eusynchytrium*.

Synchytrium cellulare. Since this fungus occurs on species of the Urticaceae also, the author was anxious to compare it with *S. chamaedryoides* with the object of determining if the two might be related. Davis (1919) described this species first on *Boehmeria cylindrica* in Wisconsin and later (1922) on *Lycopus uniflorus* and *L. americanus* also. The herbarium specimens at the University of Wisconsin show that he collected it on *L. virginicus* also. In 1922 he found a species on *Laportea canadensis* to which he gave the name *S. pulverum*, but in 1932 he observed that it develops summer sori and sporangia similar to those of *S. cellulare* on *Boehmeria cylindrica*. Therefore, he merged the two species and reduced **S. pulverum** to a synonym of the former species. Subsequently, *S. cellulare* was reported by Greene (1940) on *Pycnathemum virgianum* in Wisconsin.

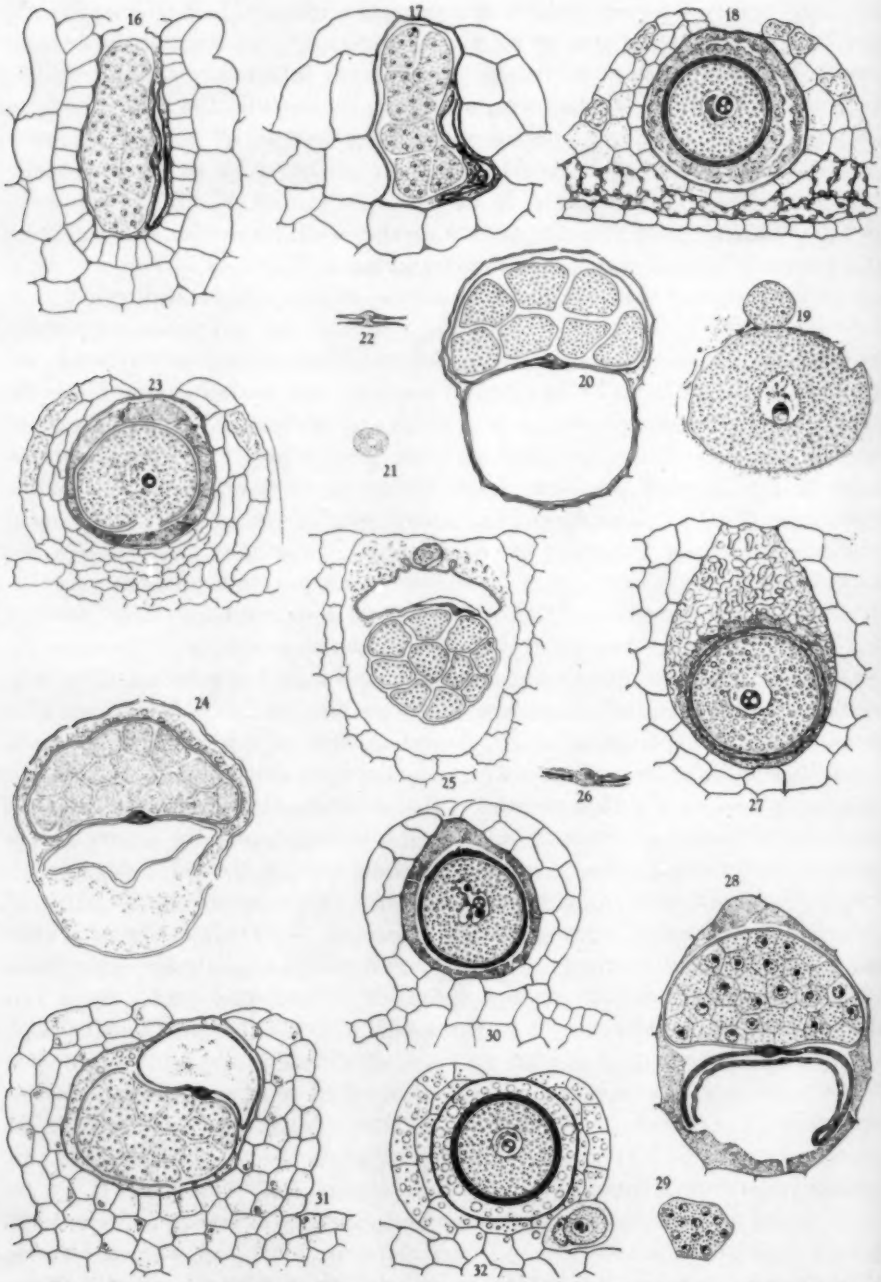
Davis' (1919) illustration and report that the infected cell is "divided by a horizontal septum into an empty basal cell and a larger upper cell which contains the summer sporangia" suggested that the basal cell might be an empty prosorus like the one described by the author (1955b) in *S. ranunculi*. This was confirmed by a letter from Dr. Myron P. Backus of the University of Wisconsin who stated that sections of the sporangial galls which he was using in his mycology class showed the presence of a prosorus and stages in the formation of the sorus. He loaned these preparations to the author for study, and at the same time Dr. H. C. Greene contributed specimens of his and Davis' herbarium material. The present study is, accordingly, based on such slides and herbarium material, and the author is very grateful to these Wisconsin mycologists for their generous contributions.

In these slides the prosorus lies in the base of the infected cell and is subspherical, 48–70, to oval, 46–78×60–$91\ \mu$, in shape with a thin hyaline wall. Figure 19 shows a prosorus from which the protoplasm is beginning to migrate or flow out to form the incipient sorus. The latter is only a small globule in this drawing, but as migration of the protoplasm continues the sorus enlarges and finally fills the upper portion of the infected cell. Usu-

ally, the empty prosorus does not collapse or invaginate but occupies the lower part of the host cell as an almost hemispherical to slightly angular cavity. Consequently, at this stage (fig. 20) the infected cell appears to be bilocular with a horizontal septum across the middle, as was shown so clearly by Davis. In this species also a plug or disk of densely stainable material is present in the region formerly occupied by the exit pore between the prosorus and sorus. It may be subcircular, 6–8 μ, or oval, 6–7 × 8–9 μ, in surface view (fig. 21) with a small pore in the center. In side view it frequently has the appearance shown in figure 22.

Davis reported that the resting spore galls are simple and unicellular, but the author found that occasionally they may be composite and multicellular also. Possibly, they vary to the same degree in this respect as reported by the author (1954a) for *A. australe* (*S. modioliensis*). Both the sporangial and resting spore galls may be very abundant on the hosts, and as they mature, dehisce, or dry out they give a tan to brown and black color to the infected portions of the leaves. In most of Davis' herbarium specimens there is no malformation, puckering, crinkling or distortion of the infected leaves. However, the flower buds of *Lycopus uniflorus* are distorted and abortive, and the galls on the stems are lavender-red in color. The general appearances of the infected portion of the hosts of *S. cellulare* are different from those infected by *S. chamaedryoides*, and the galls induced by the former species are more superficial and protruding. The prosori, sori and resting spores are essentially similar in size and shape in both species, but the sporangia of *S. chamaedryoides* are considerably larger. Also, its resting spores are usually enveloped by a denser and thicker layer of residue and have a thinner wall. In *S. cellulare* the empty prosorus lies beneath the sorus, whereas in *S. chamaedryoides* it usually occurs in the apex of the infected cell and only occasionally lies at the side or below the sorus. The most striking difference, however, appears to be the effect of infection by potential resting spores. Those of *S. cellulare* almost always cause only cell enlargement, so that the resting spore galls are unicellular and simple. The potential resting spores of *S. chamaedryoides* cause cell division as well as enlargement with the result that such galls become multicellular and composite. Whether or not such differences would occur when both species are grown on the same host remains to be seen from cross inoculation experiments. The author (1953b) attempted to transfer *S. chamaedryoides* to *Urtica dioica* without success, and it may be that this species has a very limited host range.

It is not certain that the parasite which Greene collected on *Pycnanthemum virginianum* is identical to *S. cellulare*. In fixed and stained sections of herbarium material the resting spore galls were fairly large, 132–160 μ high by 192–220 μ broad (fig. 23), partly embedded in the host tissue, and



FIGS. 16–18. *Synchytrium chamaedryoides.* FIGS 19–22. *S. cellulare.* FIGS. 23 & 24. *S. cellulare* (†). FIGS. 25–27. *S. boerhaaviae.* FIGS. 28–30. *S. oxalydis.* FIGS. 31 & 32. *S. stachydis.* FIG. 16. Embedded, crowded, elongate sporangial gall on stem; empty

all composite and multicellular. In *S. cellulare* they are predominantly simple and unicellular, as noted above. How significant taxonomically this difference may be is not certain because it is a reaction of the host to infection. Possibly, Greene's fungus might induce simple galls on the other reported hosts of *S. cellulare*. On the other hand, it is equally plausible that the structure and composition of the resting spore galls will remain largely constant regardless of host. This is suggested, at least, from the author's (1945a,b) study of resting spore galls of *S. australe* on various malvaceous and non-malvaceous hosts.

The sporangial galls induced by Greene's fungus are composite also, broadly to narrowly crateriform at the apex, 96–120 μ high by 144–160 μ broad with a sheath 1–3 cells thick. As in *S. cellulare* the initial cell functions as a prosorus and gives rise to a sorus in the upper portion of the infected cell (fig. 24). However, in most of the galls examined the prosorus had collapsed so that the infected cell did not appear bilocular as in *S. cellulare*. This may be partly due to the flattening of the galls during pressing and drying of the herbarium specimens. The mature prosorus is larger than in *S. cellulare*, subspherical, 60–72 μ, to oval, 58–65 × 70–82 μ, with a thicker, 2.8–3 μ, wall and enveloped by a greater amount of protoplasmic residue. The sorus is usually almost hemispherical, 43–52 × 63–110 μ, or slightly reniform with 2–2.3 μ thick wall. The sporangia are numerous, up to 75 in the larger sori, predominantly polyhedral, 11–22 μ in greatest diameter, and smaller than those reported for *S. cellulare*. However, this difference may be due partly to shrinkage during drying of the specimens. The resting spores are usually solitary, occasionally two in a cell, oval, 60–65 × 72–80 μ, subspherical, 68–85 μ, with a 3.5–4.8 μ thick smooth wall and coarsely granular content. They are usually enveloped by a thick and dense layer of residue which may fill the remainder of the infected cell.

Synchytrium boerhaaviae. Stevens (1927) reported this species on

prosorus at side of sorus. × 288. FIG. 17. Isolated sporangial gall with greatly enlarged sheath cells. × 171. FIG. 18. Isolated resting spore gall on leaf with resting spore enveloped by a thick layer of residue. × 230. FIG. 19. Early stage in migration of protoplasm from prosorus. × 340. FIG. 20. Infected cell with sporangial sorus in the apex; empty prosorus lining lower portion of infected cell. × 376. FIGS. 21 & 22. Plug in cross and longitudinal sections. × 599. FIG. 23. Isolated resting spore gall with resting spore enveloped by thick layer of residue. × 254. FIG. 24. Infected cell with collapsed prosorus below sorus of sporangia. × 370. FIG. 25. Embedded sporangial gall with degenerating host nucleus and empty prosorus above sorus of sporangia. × 257. FIG. 26. Plug in longitudinal section. × 600. FIG. 27. Resting spore gall with infected cell filled by residue and the resting spore. × 257. FIG. 28. Infected cell with sorus and empty prosorus; cleavage segments or protospores uninucleate. × 269. FIG. 29. Single multinucleate sporangium. × 560. FIG. 30. Isolated, protruding resting spore gall with infected cell filled with the resting spore and residue. × 204. FIG. 31. Somewhat dome-shaped sporangial gall with empty prosorus in apex of infected cell. × 170. FIG. 32. Small isolated compound resting spore gall and numerous globules in infected and adjacent sheath cells. × 269.

Boerhaavia erecta in Costa Rica and described its resting spores, sori and sporangia. Herbarium specimens and fixed and stained sections of this species at the University of Illinois were kindly loaned to the author by Dr. Leland Shanor for a study of the types of galls induced on its host. In examining the slides, which presumably were prepared by Stevens, evidence of the presence of a prosorus was found in the sporangial galls. Migration stages of the protoplasm from the prosorus are not present, but in all sections of sporangial galls an empty, collapsed vesicle occurs in the apex of the infected cell and above the sorus of sporangia. As shown in figure 25 this vesicle has the appearance and structure of an empty prosorus. Furthermore, in many sections a dense, differentially stained disk or plug of material is present at the point of contact of the vesicle with the sorus beneath. In some sections this plug is quite broad (fig. 26) and resembles the one illustrated by Rytz (1907, fig. 12) for *S. succisae*. From such evidence the author concludes that the initial cell *S. boerhaaviae* functions as a prosorus as in all of the other species described above.

Stevens did not describe the galls induced by this species, but in his slides the sporangial and resting spore galls were found to be composite and multicellular. These occur on both surfaces of the leaves, but are most abundant on the upper surface. They are also abundant on the petioles and branches, and the heavily infected, young and unfolding leaves of the herbarium specimens are malformed and stunted. Both types of galls may be single and scattered or aggregated and confluent, and are usually embedded in the host tissue as shown in figures 25 and 27. The sporangial galls are usually cup-shaped in outline and almost flat on the top with a considerable portion of the infected cell exposed. In Stevens' sections they vary from 120–140 μ broad by 108–170 μ high. The resting spore galls are pre-dominantly pyriform in shape, 78–162 μ broad by 105–192 μ high. Occasionally, two resting spores are presented in one cell, and all spores so far observed are enveloped by a dense, thick layer of residue which sometimes may fill the remainder of the infected cell (fig. 27). This contrasts sharply with infected cells with sori which contain only a small amount of residue in the apex of the cell (fig. 25). The walls of the sheath cells and infected cells are quite thin in both types of galls, and no thickening, lignification or differentiation of the wall is evident.

Synchytrium oxalydis. Cook (1947) found this species on *Oxalys repens* in Louisiana and gave a brief description of the galls, sori, sporangia, and the effects produced on the host. However, he did not note the occurrence of prosori and resting spores, and his measurements of the sori and sporangia were incorrect. In his slides of fixed and stained sections these are much larger than reported, and, in several sections prosori and resting spores are present. No migration stages of the protoplasm from the prosorus are pres-

ent in these slides, but mature as well as empty prosori are abundant. Figure 28 shows an empty one in relation to a sorus of sporangia, and here again a plug or area of densely stainable material is present in the region formerly occupied by the exit pore between the prosorus and sorus.

The mature prosorus is subspherical, 85–106 μ to oval, 65–76 × 80–96 μ, in shape with a 2.8–3.6 μ thick wall, and is usually surrounded by a dense, more or less homogeneous layer of residue which may often fill the remainder of the host cell. The sori are oval, 54–66 × 60–96 μ, subspherical, 60–160 μ, or almost hemispherical with a 2.2–3 μ thick wall. The sporangia are numerous, up to 100 or more in the large sori, predominantly polyhedral in shape, small and 12–18 μ in greatest diameter. The resting spores are spherical, 65–90 μ, or oval, 62–76 × 80–98 μ, with a 3–3.5 μ thick, smooth wall and coarsely granular content (fig. 30), and are usually enveloped by a dense layer of residue which may fill the rest of the host cell.

Several sori in Cook's sections were filled with closely-packed uninucleate segments (fig. 28) between which the lines of cleavage were quite distinct. This indicates that cleavage in the sorus of *S. oxalydis* continues or proceeds further than in most species until only uninucleate segments are present. In this respect cleavage is similar to that reported by Harper (1899) for *S. aecidioides* in which uninucleate protospores are formed. Following completion of cleavage the protospore nucleus divides, and the protospore develops into a multinucleate sporangium (fig. 29).

As Cook's photographs show very clearly, this species induces a marked reaction in its hosts. The stems and petioles become greatly thickened and malformed so that they may consist only of an irregular mass of tissue. The cellular reactions seem to be very similar to those of susceptible potato varieties infected by *S. endobioticum*, although division of the infected cell has not been reported. In addition to causing a marked enlargement of the infected cell *S. oxalydis* apparently stimulates the healthy cells to divide, whereby the infected cells containing prosori, sori, and resting spores are overgrown and become buried in the masses of thin-walled parenchymatous tissues. Occasionally, discrete galls (fig. 30) are formed which protrude prominently on the surface of the infected tissue.

Synchytrium stachydis. Cook found this species of *Stachys agraria* in Louisiana in 1945 and gave a brief description of its galls and sori. Later (1945b) in the same year he described the structure of the galls, but he did not distinguish between the sporangial and resting galls nor report the occurrence of prosori and resting spores. It is surprising that he did not observe these two developmental phases because they are very abundant and conspicuous in his fixed and stained sections which he kindly sent to the author shortly before his death. The occurrence of prosori, sori, sporangia and resting spores shows that *S. stachydis* is a long-cycled species which

possibly belongs in the subgenus *Microsynchytrium*. The present observations are based on a study of Cook's slides as well as herbarium material which he distributed, and are presented herewith to supplement his description of the species.

The mature prosorus is subspherical, 60–80 μ, or oval, 70–84 × 90–102μ, with a 2–2.6 μ thick wall, and lies above, below or at the side of the sorus in the infected cell when empty and collapsed. There does not seem to be any regularity in its position relative to the sorus in Cook's slides. Figure 31 shows a small sorus of sporangia in a large gall with the empty prosorus above. The plug of densely stainable material in the former exit pore is quite conspicuous and easily recognizable as such. In this and other sections the prosorus and sorus are partly surrounded by a small amount of protoplasmic residue. In small galls on the stem this residue is in the form of large to small and dark to almost black globules. Such globules may occur also in the sheath cells as is shown in Cook's (1945c) fig. 11a. Similar globules are abundant in the sheath cells of some resting spore galls (fig. 32), while in others they may be absent. The sporangial sori vary considerably in size and shape and may be subspherical, 66–90 μ, oval, 50–78 × 104–144 μ, sometimes almost hemispherical, 60–75 × 90–102, or slightly reniform, with a 1.8–2.3 μ thick wall. The sporangia are numerous, up to 150 or more in large sori, small, predominantly polyhedral, and 12–28 μ in greatest diameter, with a thin hyaline wall. The resting spores are usually solitary, occasionally up to 3 in a cell, spherical, 72–85 μ, or oval, 65–80 × 70–95 μ, with a smooth, 3.6–4.2 μ thick wall, and coarsely granular content (fig. 32).

The sporangial and resting spore galls are fundamentally similar in being composite and multicellular in structure and approximately the same in size and shape. The incipient sheath cells may be infected occasionally with the result that some galls are compound (fig. 32). The single and isolated sporangial and resting spore galls are largely superficial and protrude conspicuously on the host, subspherical or oval in shape, 120–180 μ high by 150–320 μ broad with a broadly crateriform apex and constricted base (fig. 32). Others may be somewhat dome-shaped with a broad base (fig. 31). When closely crowded their shapes may vary markedly, and some galls may be partly submerged in the host tissue. The sheath may be 2-4 cells in thickness and is composed of thin-walled cells which may be markedly enlarged in comparison with normal cells. No thickening and lignification of such host cells is visible in Cook's slides. The aggregated and confluent galls lead to a marked thickening, distortion, and crinkling of the leaves.

The discovery of prosori in the life cycles of these eight species of *Synchytrium* has a direct bearing on their classification in the subgenera

of the genus. Until this time they seemed to be members of the subgenus *Eusynchytrium* in which the initial cell is transformed directly into a sorus of sporangia. The presence of a prosorus in their life cycles, however, shows that they belong either in *Microsynchytrium* or *Mesochytrium*. Although their exact classification will not be possible until it is is known how their resting spores germinate, the author is assigning them provisionally to *Mycrosynchytrium* on the grounds that the resting spores of several similar species have been found to function as prosori during germination. This restudy of previously known species of *Synchytrium* indicates clearly the importance of cytological studies to proper classification, and the author believes that such studies will reveal the presence of prosori in other species in which they have been overlooked.

SUMMARY

1. The initial cell of *Synchytrium perforatum*, *S. valerianellae*, *S. holwayi*, *S. chamaedryoides*, *S. cellulare*, *S. boerhaaviae*, *S. oxalydis* and *S. stachydis* functions as a prosorus which gives rise to sorus of sporangia.

2. The occurrence of a prosorus in the so-called summer cycle of these species indicates that they belong in either *Microsynchytrium* or *Mesochytrium*, depending on the manner of resting spore germination.

3. *Synchytrium perforatum*, which was named by Ellis, is described and diagnosed for the first time.

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New Botanical Problems in the New Jersey Pine Barrens*

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My objective is to show how new techniques developed by the foresters for the management of the forests of the New Jersey pine barrens have introduced new problems for the botanists, and the peculiar sequence of events that has brought many of them to my attention. It is the common experience of all of us who are working on research of one sort or another that as we tackle one small problem new problems constantly appear. Our horizon grows and expands in all directions. And so it has been with our research in the New Jersey pine region. Specifically, I shall trace the origin and evolution of the Pine Region Hydrological Research Project. Not only has it been intimately tied up with the silvicultural developments there, but the circumstances of its growth have successively revealed a number of problems, many of which are not directly related to the specific objectives of the project. It has also created experimental areas ideally suited to the study of these problems.

We started out with one small very circumscribed problem. I use the plural WE because from the start this has been group work. John Cantlon and I started out together. He made a comment at that time that two ecologists are better than one, and I agree with him. With such a team the whole is more than the sum of its parts. The thinking of one stimulates that of the other, and the work of one stimulates that of the other, so that the result is much more than two functioning separately. The growth of the Pine Region Hydrological Research Project throughout reflects this, since it is a product not only of two ecologists, but the combined thinking of scientists of several different disciplines.

In 1946 when I came to Rutgers University Dr. Marion Johnson gave me my first introduction to the famous pine barrens. It was an exciting trip. In spite of a long history of botanical interest in New Jersey and many published papers, it seemed to me that there were many aspects of the pine barrens that had never been mentioned in the literature.

When John Cantlon came to New Jersey, he and I decided to get to know the pine barrens vegetation first hand. To us the logical approach was to work in detail in vegetation segments of limited scope. We chose to work in stands that had been long protected from fire, and these the foresters (Silas Little, Horace Somes, and J. P. Allen) helped us to locate. We chose two, one dominated by pine and the other dominated by oak. Our choice was influenced by the prevailing concept of the role of fire in the pine barren vegetation. We had learned through reading and through conversation with the foresters that the dominance of pine is the result of frequent fires; that where fires are prevented long enough, the oaks become dominant. It was our own opinion that detailed study of a mature pine stand and a mature oak stand might test this idea. It did, and we found ourselves in agreement with the concept of fire as a major factor in preventing the oaks from taking the place of the pines, at least in the area where we studied (Buell and Cantlon 1950).

A second objective that we had was to test a new method we had in mind for studying forest vegetation. Dr. Penfound (1945) had recently shown that of all quantitative information about vegetation, cover (aerial spread) is the most reliable and generally the most valuable. And Bauer (1943) had developed an excellent method of measuring cover in shrub vegetation in California by using a line transect. Wells (1946) had adapted the method to shrub-bog vegetation in the southeast. We decided it would

* Address of the retiring president of the Torrey Botanical Club, presented at the Annual Meeting, January 18, 1955.

be simple to apply Bauer's line intercept method by developing an instrument which would project the line into the canopy of the forest. The pine barren forest turned out to be an excellent place to test this method.

This initial research gave us our first intimate contact with the pine region. It gave us some understanding of the vegetation and a method well suited for work in that vegetation. Furthermore, it initiated a valuable association with a group of research foresters, an exceptionally able group of applied ecologists, including Silas Little, E. B. Moore, J. P. Allen, H. A. Somes, and George Moorhead. These men are all associated with the development of procedures, based on sound ecological principles, for managing the pine barrens forest.

To test the use of fire in managing pine barrens forest, a joint project was started in 1937 by state and federal foresters. Eight pairs of 5-acre plots were established and subjected to different frequencies of winter burning—annually, and every 2, 3, 4, 5, 10 and 15 years, in addition to a pair of control plots that were not burned at all. The objective was to produce a good pine forest. The reasoning behind the experiment was that since fire is responsible for the maintenance of pine dominance in the pine region, then the substitution of light winter fires for severe wild fires, which come at bad fire seasons, might make it possible to create superior pine forests of relatively high productivity. The present pine forests are generally of widely spaced, poorly formed trees and are of low productivity. The experiment was eminently successful.

Certain contrasting features in the ecology of the pine and the oak made the silvicultural use of winter fires effective. Fire favors the native pines (pitch pine and shortleaf pine), since pine reproduction thrives on an exposed mineral seed bed which results from the burning of the litter. The oaks are favored by litter on the forest floor. Also the pine is relatively fire resistant, the oak is much less so. Furthermore, the burning of the forest litter with creeping fires in the winter consumes the fuel that would normally feed the fires during the bad fire seasons (Little and Moore 1949).

The foresters have convincing evidence that severe wild fires can be prevented. They have also shown very definitely that an excellent pine forest can be produced this way (Little 1946 and 1952, Little, Allen and Moore 1948, Little and Moore 1945 and 1949, Somes and Moorhead 1950). Although they are aware of the rest of the vegetation, their focus is, understandably, on the trees.

What happens to the ground cover—the shrubs, herbs, mosses, and lichens? John Cantlon and I were permitted to work on the experimental plots with the objective of finding an answer to this question. We found some very interesting things. The most striking was the very pronounced decrease in ground cover resulting from a reduction of the extremely high natural shrub cover. Various species reacted quite differently. Huckleberry (*Gaylussacia baccata*) decreased greatly. The low bush blueberry (*Vaccinium vacillans*) was so much less affected by the burning that its importance in the resulting vegetation is proportionately greater. Mosses and lichens increased rather remarkably. When the trees were cut *Carex pensylvanica* grew in profusion, resulting in a "grassy" aspect (Buell and Cantlon 1953).

These changes are really very pronounced and, along with the increase to maximum pine cover in the canopy, result in a drastic change in the vegetation.

Botanically the results are interesting in that our data indicate that no species is eliminated as a result of the burning. This is perhaps of particular significance. Apparently the long fire history of the pine region has long ago destroyed any species that cannot live through forest fires. Weaver and Clements (1929) called the pine barrens vegetation of New Jersey a fire subclimax, and in the dictionary of Clementsian terminology this seems to be the proper term for it.

We began to realize that such a drastic change in the vegetation must greatly affect the amount of water that gets into the ground. In the summer of 1950 we had a chance to study the relation between vegetation and water. Although we did not realize it at the time, this was the initiation of a project that was destined to grow. A peculiar set of circumstances led to our study that summer.

John Cantlon and I wanted to do further work in the pine barrens. The foresters

were willing to have us use their experimental plots for our work. Most important was the interest that Mr. Charles Doehlert of the Cranberry and Blueberry Research Laboratory of Rutgers University took in our work and through him the cranberry and blueberry growers, who were willing to support research in that area. They asked us to define a project we should like to work on. We decided we would like to make a study of rainfall interception under pines and under oaks and under a combination of oaks and shrubs. This was, of course, thinking in terms of the broadest effects of the silvicultural practices. It was a project that fascinated us, and of course, it did the cranberry growers too because they have a real interest in the local water supply. They gave us $300, which made it possible for John Cantlon to spend his summer on the project.

The results of Cantlon's studies were not conclusive. His time and equipment were both limited. But he had rain gauges—some standard, some specially made trough gauges, and some fashioned out of 5-quart oil cans. He placed reference gauges in the open. The others he placed under trees, and under shrubs and trees combined.

His results (Cantlon 1951), indicating the pines as more efficient interceptors of rainfall than the oaks, are in contrast to Kittredge's (1948) generalization that the nearer the vegetation to climax, the greater the interception loss. Cantlon's data showed the pines as intercepting 28 percent of the rainfall of the season, the oaks 15 percent, and a combination of shrubs and oaks 23 percent. One would infer from this that even with the shrubs included, the oak vegetation is less effective than the pine in intercepting precipitation. But this statement is misleading. One must take into account two things—that the scope of the study was limited in time and equipment, and it ignored the influence of the heavy layer of oak litter on the forest floor of unburned areas. No one has yet worked on litter interception in the pine barrens, and very little such work has been done elsewhere. Also no study of stemflow has been made.

Mosses and lichens can also absorb appreciable amounts of precipitation. Dr. E. T. Moul and I (1955) found that burning induces the increase of these plants up to 30 percent or more of cover, and where they are present they may absorb up to 1.5 inches of rain in any one storm. To fully evaluate the relative importance of this layer, again one would need to know the interception value of the litter layer whose destruction makes possible the moss and lichen mats.

During the course of John Cantlon's research on interception in 1950 an incident which broadened our horizon considerably occurred. Mr. Henry C. Barksdale visited the project. He is the District Engineer for the Ground Water Branch of the U. S. Geological Survey. Not only is he a man accustomed to thinking in terms of large bodies of underground water, but he is a man of remarkable vision and foresight. He spoke in terms of a great underground reservoir underlying the two thousand square miles of pine barrens. Below the water table and extending to a great depth, up to 20 percent of the volume of the coarse sediments is pore space filled with water. It is New Jersey's last essentially untapped source of water. It is a remarkable resource, since the volume of storage available will permit withdrawal in the excess of replenishment in years as well as months of low precipitation and its subsequent replenishment in months or years of high rainfall. It could yield at least a billion gallons of water daily without depletion.

Mr. Barksdale (1952) visualizes withdrawing this water through properly dispersed wells throughout the area, much as oil is removed from oil fields. No dams with large surface reservoirs are necessary or even desirable. He translates inches of rainfall into billions of gallons of water, and when one stops to realize that if two types of vegetation differ in their interception and use of rainfall by 5 percent, or somewhat over 2 inches, that adds up to a lot of water over an area of two thousand square miles. So John Cantlon's preliminary studies on interception indicate that an enormous volume of water may be involved as vegetation changes.

With Mr. Barksdale we planned a project whereby two watersheds would be set aside as research areas. On one the forests were to be managed by the new silvicultural procedures of prescribed burning but the other would not be so handled.

It was to be a co-operative project—the management of the forests would be in the hands of the New Jersey State Foresters and the personnel of the U. S. Forest Service; the measurement of the vegetation changes would be done by Rutgers botanists; determination of the effect upon the potential water yield of the area was to be the responsibility of the Ground Water Branch of the U. S. Geological Survey. It was to continue over a period at least long enough to follow through changes associated with the growth and harvest of a crop of pine.

To initiate the project it was first necessary to enlist the support of the New Jersey Department of Conservation and Economic Development. This department includes the Bureau of Forestry, Parks and Historic Sites. Not only does it administer the Lebanon State Forest where the two watersheds we selected are located, but also it includes the foresters who will manage the forests of the watersheds. The department also includes the Division of Water Policy and Supply, which of course is the primary state agency interested in water problems.

Secondly, we needed to raise funds locally (in New Jersey) to support research on the initial phases of the vegetation work. The several sources of contribution indicate the rather general interest in and recognition of the value of the project: (1) American Cranberry Growers Association, (2) Growers' Cranberry Co., (3) New Jersey Blueberry Farmers' Association, (4) Atlantic County Board of Agriculture, (5) Burlington County Board of Agriculture, (6) Hammonton Co-operative Fruit Auction Association, (7) Miss Elizabeth C. White, (8) The Flintkote Company, (9) The Barrett Division of the Allied Chemical and Dye Corporation, (10) The Southern New Jersey Development Council, (11) The New Jersey Department of Conservation and Economic Development, and (12) Rutgers University. The contributions have been sufficient to support a research fellowship at Rutgers University and the expenses associated with it. This local support was exceedingly important, because the Ground Water Branch of the U. S. Geological Survey could not establish and assign funds to such a project unless a local group invested an equivalent amount in it.

Field work on the Pine Region Hydrological Research Project started in the fall of 1951 with Mr. Jack McCormick of Rutgers University handling the vegetational work and Mr. Edward C. Rhodehammel of the Ground Water Branch of the U. S. Geological Survey handling the field work of the problems related to the ground water. Much of the work done so far has been on common ground, however, such as delimitation of the watersheds, establishing a grid system, and monumenting it with concrete markers. Jack McCormick has worked out methods for measuring the vegetation and has completed the initial ecological survey. Drilling throughout the watersheds to determine the detailed near-surface geology has been started. Some wells for observing groundwater conditions have been established. A system of precipitation stations has been set up to determine the rainfall pattern. Mr. O. W. Hartwell of the Surface Water Branch of the U. S. Geological Survey has been supervising the erection and maintenance of two stream gauging stations for the project, one on Middle Branch and the other on McDonald's Branch, the two watersheds being used. In other words, the project is now well started. A period of calibration of the watersheds will be necessary before the treatments are begun.

New botanical problems of the New Jersey pine barrens arise out of the prescribed burning, which in turn stimulated this project. Also this project itself emphasizes the importance of learning more about plant-water relations, particularly water use and interception by vegetation. Numerous problems introduced by the burning practice were enumerated in a paper presented by Dr. Cantlon and myself (1952) at a symposium organized by Dr. John A. Small at Philadelphia in 1951 (Small 1952). The type of problems that we emphasized there were those that particularly relate in one way or another to the commercial blueberry and cranberry crop, such as disease problems, pollination problems, and water supply problems. Important also is the relationship between vegetation and wild life—the study of plants both as food source and cover. But in addition, there are many others of primarily academic interest, as for example, why are some plants less susceptible than others to burning. And then of course there

are many old problems that have never been settled, problems that were being discussed long before prescribed burning was thought of.

In spite of many years of botanical activity in the pine barrens, we have scarcely scratched the surface. A lot has been done it is true, but much opportunity lies ahead. Many old problems still exist untouched, but new approaches have been introduced, and with them the scope of botanical research there is broadening. The two watersheds that have now been set aside are a unique laboratory, ideally suited for the study of many of these problems.

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CYPRIPEDIUM ACAULE

Thousands of pink lady slippers bloom every May in Bergen County, New Jersey,

only three quarters of a mile from the center of a small town and perhaps 500 yards from a new housing development, yet oddly secluded enough to prosper. This remarkable stand bordering the lake on the reservation of the Hackensack Water Company at Emerson came to the attention of the Garden Club of Englewood several years ago. The Club decided to make a study of the area in 1954, because of its interest in conservation. Mrs. Pell W. Foster, Jr., and I agreed to undertake the project, substituting enthusiasm for scientific knowledge.

The *Cypripedium acaule* grow under white, red and scotch pines through a bed of pine needles two inches deep. The soil is sandy, the result of dredging. Associated plants are spotted wintergreen (*Chimaphila*), rattlesnake plantain (*Epipactis*), virginia creeper (*Psedera*), poison ivy (*Rhus*), blueberry (*Vaccinium*), bayberry (*Myrica*) and horse tail (*Equisetum*).

The location was visited regularly beginning April 1st twice each week during the growing season and after May 25th at varying but frequent intervals until frost, covering a period of six months.

Cyprepedium acaule, Bergen County, New Jersey, May 1954.

The first lady slippers were evident on April 18th. The new shoot grows from the old crown next to last year's stalk and seed pod, many of which still stand. Tightly folded leaves unfurl when 4–7½-inches high. The flower bud forms with the leaves and is noticeable to the touch when the leaves are one inch above the bed of pine needles. The outside leaf separates, the green flower bud within the tightly closed second leaf emerges and it grows very rapidly. As the bud develops the bract becomes erect. At first the sepals are pinky green, the sac or pouch is greenish white. As the sac swells, it turns pink, the sepals darken to purplish brown. When the flower reaches maturity, the color intensifies and the pouch becomes prominently veined. May 18–20 was the peak of bloom in 1954.

The lady slipper, a monocotyledon, has two large basal elliptical leaves deeply creased by veins. These are borne above a cluster of white fleshy roots. From the center

of these pairs of leaves which measure 8–10 inches long and 3–5 inches wide, arises a naked scape, varying in height from 3–19 inches. At the summit is the flower surmounted by a small leaf-like bract. The flower consists of three lanceolate sepals which are green shaded with purple; there are two lateral, lanceolate petals which are flat, hairy, twisted and slightly longer than the sepals and darker in color. The drooping inflated sac is pink or roseate, oblong, very veined with a fissure extending down the front. The upper part of the lining is covered with hairs. At the base of the lip is found the column which is the coalesced style, and filament. The pollinia, plainly visible either side of the lip are yellow, sticky, granular masses. At the base of the flower stalk, within the two basal bud bracts, may be found the bud which will produce next year's leaves and flower. New plants grow from seed and require several seasons growth before flowering.

In this stand of lady slippers, the first flower faded on May 25th. The ovary develops very quickly into an oblong green seed pod, pointed at both ends. The majority of plants dropped their flowers without forming pods, leaving a single bract on the stalk. The green seed pods remained outwardly unchanged all summer. A killing frost occurred on September 30th and again October 1st. We observed on October 6th that the pods had started to turn brown and were splitting along the ribs to release the seed. The seed is the color of white pepper, fine, powdery and slightly granular.

A count taken of the density of plants per sq. yd. in one plot revealed 46 plants, 34 flower buds; in another plot 40 plants, 24 flower buds; in another 57 plants, 13 flower buds where there was a large percentage of seedlings. An area approximately 15×20 feet showed 194 plants. Outstanding clumps consisted of 8 plants, 8 flowers; 11 plants, 10 flowers. On these three or four acres which we studied there were certainly some thousands of flowers. During the growing period April 18–May 18, there were 13 sunny days, 11 cloudy days and 16 rainy days.

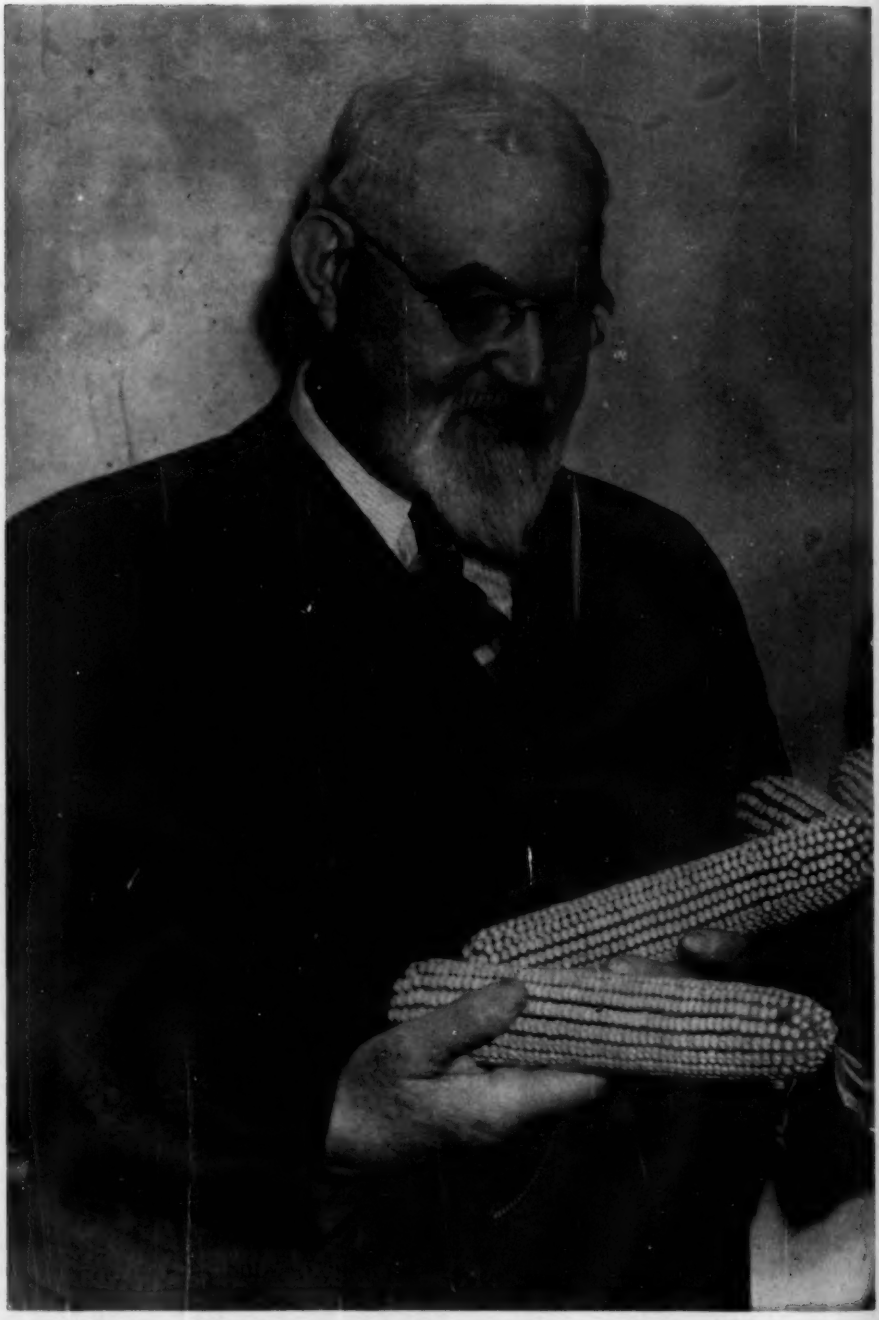
The exquisite beauty of these plants in full bloom is indescribable. Great masses of exotic pink flowers nodding in the breeze presents an illusion of remoteness and unreality. To see them growing in their natural habitat, undisturbed and in fantastic numbers gives one some conception of the fabulous luxuriance of the wilderness that existed before civilization took its toll.—Lydia M. Seeley.

George Harrison Shull

Dr. George Harrison Shull, late Professor Emeritus of Botany and Genetics at Princeton University, discoverer of hybrid corn and a former president of the Torrey Botanical Club, was born on a farm near North Hampton in Clark County, Ohio, on April 15, 1874. He was the son of Harrison and Catherine Ryman Shull and was one of seven children all of whom became prominent educators. Dr. Shull and four of his five brothers were at one time listed in Who's Who in America and he and three of the brothers were once included in American Men of Science.

Dr. Shull's early formal education was not very extensive. His parents were not well to do and as a boy he lived on eight different rented farms. His life was strenuous. When he was small, he lived too far from school to attend during the three worst months of the winter. When he was older he could not go to school in the fall until the corn was gathered and he had to drop out of school about the first of March for spring planting. Consequently he never had a complete year of schooling. In his boyhood, as in his mature years, he had a great interest in learning and had tremendous drive and energy, so that he supplemented his meager formal education with extensive and intensive home study and mastered most of his high school studies in that way. Perhaps one would be more accurate to state that he supplemented his home study with some formal, classroom education.

Rural life tends naturally to bring a child into contact with nature more than does an urban background, and Dr. Shull had an early interest in learning about the plants around him. At eleven or twelve years of age he learned how to trace plants through keys and at sixteen he decided to become a professional botanist. His plans were delayed for financial reasons.



George Harrison Shull April 15, 1874—September 28, 1954

At eighteen, Dr. Shull began his career as a teacher in the county schools in rural Ohio and for five years he taught seven grades each day. In 1901 he passed an examination for Principal of the Springfield High School but, fortunately for his subsequent career, someone else got the position. At the end of his fourth and fifth years of teaching he studied at Antioch College. As he had exhausted his savings the faculty granted him free tuition and provided jobs so that he could support himself. He began his full-time study in 1897 and received his B.S. degree in 1901. His various self-supporting jobs were custodian of the laboratories and library, engineer in charge of the steam-heating plant and water works, head janitor and college plumber. He firmly believed that hard work was a character builder and that young people should be partially self-supporting. He applied these ideas to his children who, while in school and college, spent their summers as farm laborers in his Oenothera field.

Just before Dr. Shull's graduation from Antioch he took a Civil Service examination for an assistant's position in the U. S. National Museum but although he received a high grade was not appointed at the time. He did obtain a position as a student assistant in the U. S. Bureau of Forestry and spent some time surveying forests in eastern Tennessee. In 1902 his appointment to the National Herbarium came through and three months later he was transferred to the Bureau of Plant Industry and assigned to study the plant life of Chesapeake Bay. The population of wild ducks in that area was seriously being reduced and for two summers he studied whether this dimunition was caused by a reduction in the number of plants which the ducks used for food. Years later he mentioned to the writer that the problem was really a simple one. The ducks were being shot more rapidly than they could reproduce. In September, 1901, Dr. Shull entered upon his graduate work at the University of Chicago. Dr. Charles B. Davenport was teaching biometry at Chicago then, and as Dr. Shull had already made a statistical study of variation in *Aster*, this was a fortunate circumstance. The University of Chicago allowed him to use his work on place constants for *Aster prenanthoides* as a thesis and granted him the Ph.D. degree in March, 1904. Two months later he received an appointment in the newly formed Station for Experimental Evolution founded by the Carnegie Institution of Washington. In 1906 the Carnegie Institution decided to study the spectacular work of Luther Burbank and from 1906 to 1911 Dr. Shull was assigned to work at Santa Rosa, California, for half of each year. In 1915 he left the Carnegie Institution to become Professor of Botany and Genetics at Princeton University. In 1942 he became Professor Emeritus and retained that title until his death on September 28, 1954.

On July 8, 1906 Dr. Shull married Ella Amanda Hollar, but she died in Santa Rosa on May 7, 1907. He married Mary Julia Nicholl of Rochester, New York on August 26, 1909. They have had four sons and two daughters all of whom are living. At present there are 23 grandchildren. There has probably never been any more devoted a family than the Shull family. Birthdays were always a cause for celebration and the writer will always remember how Dr. Shull appeared late for his laboratory in general botany because of a celebration on his fiftieth birthday.

Dr. Shull's active scientific career covered well over half a century. His first paper was published in AMERICAN GARDEN in 1891, when he was just seventeen years old. Several months before his eightieth birthday he reported on some studies on oaks at the Boston meetings of the Genetics Society of America in 1953. In his precollege days he was interested in the development of buds in a number of different genera, and published several papers on this work. He also had some interest in the geographical distribution of *Isoetes*.

His genetic work was concerned chiefly with maize, the evening primrose (*Oenothera*), and shepherd's purse (*Capsella*, or *Bursa* as he called it since he followed the American code), but in his earlier days he carried out genetic studies on a number of different plants. This work included biometrical studies on *Aster*, oats, and *Ceratophyllum*, a study of development in *Sium cicutaefolium*, sex linkage in *Lychnis* (*Melandrium*) and studies on the inheritance of various traits and the testing of Mendel's laws in ash, poppies, beans, violet hybrids, elm, foxglove, primrose, stocks, beets, turnips, wheat,

flax, *Oxalis* and *Brassica*. Outstanding among these papers was the work in *Lychnis*, for he was one of the first to demonstrate sex linkage in plants. *Oenothera* and *Capsella* (*Bursa*) were the two plants to which he devoted most of his time. He began his work on each of them in 1904 and continued with them until his retirement, and from the time of his appointment to Princeton he worked on them almost exclusively. During that 38-year period he raised and studied about 1,000,000 *Capsellas* and around 750,000 *Oenotheras*. In the *Oenothera* work he found many mutants and obtained many data in trying to resolve the difficulties peculiar to that organism. In *Capsella* he made a study of various species, their distribution and the distribution of their genes throughout the world. In this last genus he found one of the classic cases of duplicate genes and one which has been cited in a number of textbooks. His work in *Oenothera* illustrates Dr. Shull's fine character. For a number of years he differed radically with the interpretation that Dr. R. E. Cleland was applying to a solution of the *Oenothera* problem, but throughout their controversy both the men kept their differences on a purely intellectual

The Shull family at the 30th wedding anniversary, August 26, 1939. Standing, left to right: John, Fred, Harrison, Barbara, Mungo F. Miller, Georgia, Peter R. Vandersloot, David. Seated: Bertie, Alleyne, Mary J. Shull, George H. Shull.

plane and remained the best of friends even though they disagreed very strongly in their scientific work. Throughout the dispute, too, Dr. Shull allowed Dr. Cleland to have access to all his pedigreed cultures and to study them freely. Dr. Shull was very happy in his work with *Oenothera* and *Capsella* and felt that it contributed more of purely scientific value than his work on corn.

The corn work was started in 1905, and was inspired by Johannsen's work on inbreeding in beans. This work had just been published and Dr. Shull reviewed it for the BOTANICAL GAZETTE. In maize, Dr. Shull demonstrated that repeated self-fertilization will result in the establishment of homozygous lines which will be very inferior to the original types, but that when these lines are crossed most of the combinations will be greatly superior to the inbred lines and a few will even exceed the original strains in size and yield. This hybrid vigor he termed "heterosis" in a lecture delivered in Göttingen, Germany in July, 1914. He liked to relate that during the lecture he wrote the

word on the board in German rather than English script and that the audience burst forth into applause. Obviously most of the audience were Germans and it was within a very short time of the opening of the first world war. In 1909 he suggested a practical way in which the principles he had discovered could be applied commercially, but the production of hybrid corn seed was not begun on a commercial scale until 1922 and by 1937 only four per cent of the corn acreage of the United States was planted with hybrid corn. It gained wide acceptance during the second world war, however, and is now grown on over three-fourths of the acres devoted to corn. It is valuable because it has commonly resulted in yields 25 to 35 and sometimes even 50 per cent greater than seed produced by the older breeding methods. Agriculturists have estimated that the cash value of the increased production from hybrid corn during World War II was enough to pay for the development of the atomic bomb.

In 1918 Dr. Shull organized the Genetics Section of BOTANICAL ABSTRACTS and he was its editor for four years. He founded GENETICS, the leading American genetical journal, he organized its editorial board and he was the managing editor during its first ten years (1916–25) and an associate editor from that time until his death. He was interested in terminology and introduced several terms, including "duplicate genes", "sib", "defective inheritance ratios" and "heterosis". He was the first English-writing geneticist to substitute Johannsen's "gene" for the then current "factor". He proposed "allele" for "allelomorph" and was instrumental in having that term adopted by GENETICS and he was largely responsible for the spelling of "gene", "clone", "allele" and "genome". Some biologists had spelled these words without the final e.

Dr. Shull was a member of a number of scientific societies both in the United States and abroad. He was vice-president (1911) and president (1917) of the American Society of Naturalists and was president of the Torrey Botanical Club (1947). In 1912 he was chairman of the plant section of the American Genetic Association and has been on the advisory committee of that society since 1922. Other American societies of which he was a member included the American Philosophical Society, Genetics Society of America, Botanical Society of America, Ecological Society of America, American Society of Plant Physiologists, Eugenics Society of America, Eugenic Research Association, A. A. A. S. and Washington Academy of Science. He was instrumental in organizing and was the first president of the Princeton chapter of Sigma Xi (1932–33) and since 1940 was the honorary president of the Antioch Alumni Association. Dr. Shull was a corresponding member of the Deutsche Botanische Gesellschaft and the Academy of Science of Vienna and an honorary member of the Gesellshaft für Pflanzenzüchtung in Wien and the Swedish Seed Association. He was a member of the Deutsche Gesellschaft für Vererbungswissenschaft, the Société Linnéenne de Lyon and the Institut International d'Anthropologie (Paris). His hybrid corn work brought him several honors in his later years, including the Marcellus Hartley Medal of the National Academy of Sciences in 1948, the De Kalb Agricultural Association Medal (1940), the John Scott Award of the City of Philadelphia (1946) and the New Jersey State Board of Agriculture Annual Citation for Distinguished Service to Agriculture (1945). Of all the honors he received perhaps the one that pleased him most was his selection to Popular Mechanic's Fiftieth Anniversary Hall of Fame as one of the fifty most important men of the half-century in science and engineering (1952). He received three honorary degrees, an LL.D. from Antioch College in 1940 and honorary Sc.D. degrees from Lawrence College (1940) and Iowa State College (1942).

Undoubtedly his family was Dr. Shull's chief interest and joy in life, with botany and genetics a close second, but in spite of the long hours he devoted to both, he still had time left for civic activities. From 1928 to 1944 he was a member of the Princeton Borough Board of Education. He was vice-president from 1934 to 1936 and president from then until 1944. During that sixteen-year period three new school buildings were erected and equipped and in 1939 Dr. Shull was chairman of a special committee to supervise the construction of the Princeton High School .This last position he filled with the same thoroughness and attention to detail and with the same conscientiousness and

devotion to duty that characterized everything he ever undertook. The writer was a graduate student at the time and well remembers Dr. Shull's telling him how he walked past the construction work every day on the way from his home to his laboratory and how he insisted that the contractor abide literally by every term of the contract and substitute no inferior materials or workmanship. If that high school is not well built it is not Dr. Shull's fault. Another of his civic activities was his long and hard fight to have Island Beach on the New Jersey coast between Barnegat Bay and the Atlantic Ocean kept away from a group that wanted to develop it into a recreation center and to have it placed under the National Park Service who would make it a National Monument to be preserved for future generations as a wild-life sanctuary. This area is the last undeveloped dune land in that part of the country. To preserve this area he organized the Island Beach National Monument Committee in 1945 and served as its chairman for five years. The achievement of his aims meant a hard fight against political influence but in the end the fight was successful and the region has been preserved for nature lovers.

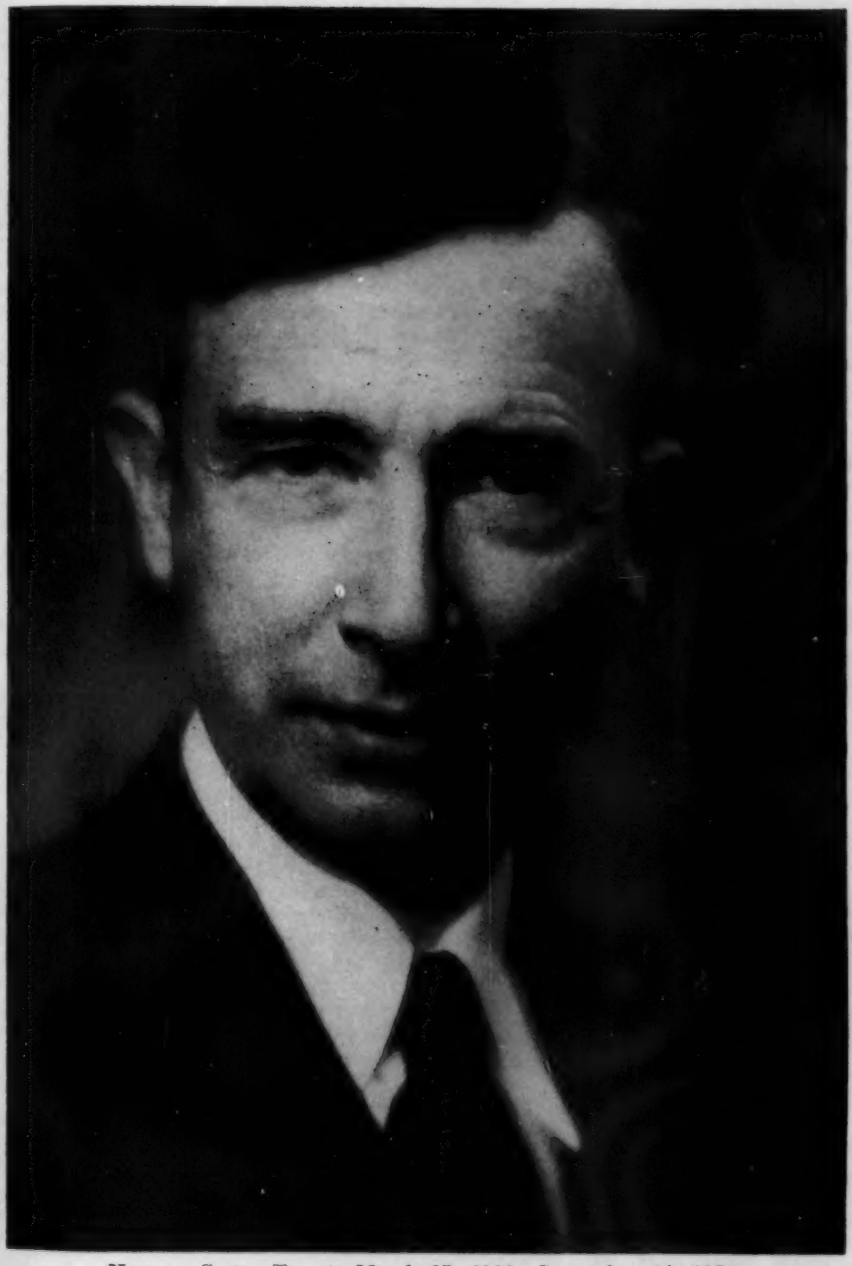
Dr. Shull was a great admirer of Dr. John Torrey. Not only was he a long-time member and former president of the Torrey Botanical Club but he founded an undergraduate botanical club at Princeton which he called the "John Torrey Club of Princeton." In a set of suggestions which he left for his executrix he asked that his reprints other than genetical, his set of botanical works of John Torrey and a bas-relief portrait plaque of John Torrey be given to the Princeton biology department with the hope that some day Princeton University would found a botanical laboratory called the "John Torrey Botanical Laboratory." Since Professor Torrey had once taught at Princeton, this suggestion is not inappropriate.—HERBERT PARKES RILEY, University of Kentucky.

Norman Carter Fassett

Norman C. Fassett came from a Boston manufacturing family but spent much of his youth in provincial New England. In spite of the Boston background, in spite of undergraduate and graduate training at Harvard and thirty years at the University of Wisconsin, he retained to the end of his life much of the speech and the point of view of the old-fashioned, small-town New Englander. He had the dry humour, the quizzical distrust of the boom-of-the-moment, the peculiar turns of speech, the complete lack of pretense, the strange combination of personal dignity and respect for his own oddities, which was notable in such diverse fellow New Englanders as Henry Thoreau and Calvin Coolidge. He would discipline his classes by telling them about Willie, the town idiot of Leominster, who played a trick on the bus company by throwing away, unused, his bus ticket after he had purchased it. "Now Willie", he said with a quizzical look, "was an idiot". Some of the students got the point of this odd introduction to a course in Botany; a few of them remembered it the rest of their lives.

He was a real teacher. After he was a full Professor he continued not only to teach elementary courses, but to spend time and thought on the laboratory exercises, seeing to it that the material was presented in such a way as to induce observation and reflection. He taught his colleagues, though they did not always understand his extra-dry New England humour. He taught their students as well as his own, in formal classes and in informal contacts. On one occasion, busying himself at putting things to right in a cupboard which happened to be in the plant physiology laboratory, one of the graduate students working there stepped up and wanted to know if he could be of any assistance. "No, no", drawled Fassett. "I saw the sign on the door. It says PLANT physiology. I was just looking around to see if I could find the plant; it doesn't seem to be here".

He was at his best with advanced students, for to them his intense enthusiasm for plants proved most contagious. Many an unsuspecting student was diverted from a casual interest in some other phase of botany, or had a generalized interest rapidly focused on Systematics by his course in "Spring Flora of Wisconsin". The appeal lay not only in the manner of presentation—the enthusiasm and sense of importance of the subject—but as much in the professional level at which it was given and the



Norman Carter Fassett March 27, 1900—September 14, 1954.

broad basis upon which the course developed. Geology, meteorology, physiology, anatomy and morphology, ecology and conservation were fused successfully into a related whole.

He was in the field with his students as much as possible and perhaps to relieve them of any delusions about the seriousness of the work, delighted to lead the way through the densest thickets and briar patches, or the boggiest parts of the swamp where the mosquitoes were largest, while the objective always lay by an easier path. There was no lecturing on fundamental concepts or methodology. Rather these were expressed, in characteristic fashion, from time to time as a fitting occasion arose. Once, when a student failed to take the underground parts in preparing a specimen, he questioned this procedure. The excuse was offered that there had never been any characters found in the roots and underground stem. He replied, "Perhaps because nobody collected them".

To emphasize the necessity of always consulting original sources he said, "Never trust anybody's work, least of all your own". There was a complete lack of dogmatism in his teaching. It was pervaded by an underlying skepticism and he delighted when new evidence upset established concepts. He enjoyed explaining, when the tamarack bogs of the state were under discussion, "I have always said there are no tamarack bogs in the Driftless Area. But now one of my students has written a thesis about them".

He taught widely outside the University, in his daily contacts with the general public. He took very seriously his job to find out about the plants of the state and collected in all parts of it tirelessly and thoroughly. This brought him in contact with many kinds of people; farmers, sportsmen, amateur naturalists, fellow scientists. To all of them he endeavored to impart some knowledge of the plant cover of Wisconsin, its history and its significance to the community at large. He was a pioneer, with Aldo Leopold, in developing a conservation program in the state. One of his major works, "The Aquatic Manual", grew out of these contacts and interests. It brought a knowledge of the kinds of water plants, previously available only to a very few experts, within the grasp of any intelligent amateur who would take the trouble to consult his clear, orderly, and comprehensive volume.

In his own conservative way he kept abreast of the times. When population genetics began to develop concepts which bore directly on some of the basic problems of taxonomy, he mastered the technical literature. Not content with taking other people's formulas on faith, he set up a model population with a bowl of different colored marbles and demonstrated to his own satisfaction the action of selection on Mendelian factors. He became interested in hybridization in natural populations and worked out his own effective methods for analyzing and reporting the complex situations he uncovered in such genera as *Rubus* and *Typha*.

His later years were clouded by a malignant brain tumour which produced disquieting physical and emotional effects long before it was diagnosed. After a drastic operation had put its finger on the seat of his difficulties and given him a brief final respite, he spent the few remaining months of his life bringing as many of his scientific projects as possible to an effective close. He faced his swiftly approaching end with no false cheeriness but with Olympian calm and met his increasing disabilities nobly and efficiently.—EDGAR ANDERSON AND ROLLA M. TRYON, JR., Missouri Botanical Garden.

NOTES

The following new members were elected to the Club during 1954. **Annual Members**: Pieter J. Bels, Box 650, Waterloo, Quebec, Canada; Professor F. H. Bormann, Biology Dept., Emory University, Emory University, Georgia; Dr. Van Montague Browne, 200 S. Drive, Miami Springs, Florida; Mr. Grant R. Dorheing, A. H. Gibson Lab., University of Pittsburgh, Pittsburgh 13, Pa.; Otto J. Ellik, 127 Willow St., Brooklyn 1, N. Y.; Edward J. Feeley, Biological Lab., Fordham University, New York 58, N. Y.; Hugh D. Freudenthal, 184 East Shore Drive, Massapequa, L.I., N. Y.; Dr. Mason E. Hale, University of Wichita, Wichita 14, Kansas; Mrs. Muriel J. Landauer, 16 Muldowney Circle, Poughkeepsie, N. Y.; Professor Addison E. Lee, Botanical Lab., University of

Texas, Austin 12, Texas; Dr. Peter K. Nelson, Brooklyn Botanic Garden, Brooklyn 25, N. Y.; Mr. Bernard C. Patten, Jr., Box 167, Braddock Heights, Maryland; Dr. John Poindexter, Bio. Dept., Occidental College, Los Angeles 41, California; Dr. Fred M. Schellhamer, 166 Rocky Rd., Larchmont, N. Y.; Dr. Jacob Straus, Biological Lab., Harvard University, Cambridge 38, Mass.; Professor Ruth I. Walker, University of Wis., Milwaukee Ext., 600 W. Kilbourn Ave., Milwaukee 3, Wis.; Dr. A. W. Ziegler, Department of Botany, Florida State University, Tallahassee, Florida.

Associate Members: Mr. Nathan Apple, 201 Clinton Ave., Apt. 11 E, Brooklyn 5, N. Y.; Miss Helen E. Berger, 44 E. 78th St., New York 21, N. Y.; Mrs. Sophie Dew, 2710 Webb Ave., New York, N. Y.; Mr. Herman Druin, Box 183, Passaic, N. J.; Mr. W. J. Highton, 30 Dunnell Rd., Maplewood, N. J.; Mrs. Catherine B. Hodkinson, 60 Park End Place, East Orange, N. J.; Miss Hazel Holly, St. Barnabas Hospital, 4422 Third Ave., New York, N. Y.; Mr. Herman Kretschmer, West Nyack, N. Y.; Mrs. Lucy G. Loekle, Allendale, N. J.; Mr. Gregg M. Moga, Spencer Arms Hotel, 140 W 69th St., New York 25, N. Y.; Mrs. John T. Neal, Greystone Park, N. J.; Mr. Charles Nichols, 654 Bergen Ave., Jersey City 4, N. J.; Mr. Leonard J. Uttal, 626 Wallelein Rd., Uniondale, N. J.

At the Annual Meeting on January 18, 1955, the following were elected for 1955. **Annual Members**: Mr. Robert B. Clark, Department of Horticulture, Rutgers University, New Brunswick, N. J.; Rev. R. J. Cronin, Spellman Hall, Fordham University, New York 58, N. Y.; Dr. David E. Fairbrothers, Botany Department, Rutgers University, New Brunswick, N. J.; Mr. Walter T. McDonough, 360 Ninth Ave., New York 1, N. Y.; Dr. Albert Schatz, Research Director, National Agricultural College, Farm School P.O., Bucks Co., Pa.; Dr. Erich E. Steiner, Botany Department, University of Michigan, Ann Arbor, Michigan; Dr. Chao Nien Sun, 3546 Caroline St., St. Louis, Missouri; Mr. Howard Swift, 955 Sheridan Ave., Apt. 1 C, New York 56, N. Y.; Mr. Fred Townsend, 16 N. Gosse Blvd., Princeton, Illinois; Mr. Leonard J. Uttal, 626 Wellelein Rd., Uniondale, N. J.

Associate Members: Mrs. Maojrie S. Boyle, Box 293, Monmouth Beach, N. J.; Mrs. Burton Dezendorf, 401 Hasbrouck Blvd., Oradell, N. J.; Mrs. Ruth M. Lynch, 200 East 26th St., New York 10, N. Y.; Mrs. Jeanette C. McCarthy, 851 Carroll St., Brooklyn 15, N. Y.

Officers for 1955 elected at the Annual Meeting were:

President	Elva Lawton, Hunter College
1 Vice-President	Ralph H. Wetmore, Harvard
2 Vice-President	Edwin T. Moul, Rutgers
Corresponding Secretary	Eleanor Witkus, Fordham
Recording Secretary	Donald P. Rogers, N. Y. Botanical Garden
Treasurer	Gily E. Bard, 82 Bella Vista St., Tuckahoe, N. Y.
Editor	Charles A. Berger, Fordham
Business Manager	Regina Duffy, L. I. University
Bibliographer	Lazella Schwarten, Arnold Arboretum

Delegate to the Council of the New York Academy of Sciences
Ralph H. Cheny, Brooklyn College

Representative on Board of Managers of the N. Y. Botanical Garden
Harold H. Clum, Hunter

Representatives on the Council of the A.A.A.S.
Murray F. Buell, Rutgers, Lindsay Olive, Columbia

Members of the Torrey Botanical Club Council 1955–57
Annette Hervey, N. Y. Botanical Garden — Joseph Schue
George McNew, Boyce Thompson Institute — David Keck, N. Y. Botanical Garden

Members of the Council 1954–56: William Crotty, N. Y. University
Regina M. Duffy, L. I. University

Members of the Council 1953–55:	Honor Hollinghurst
	Harry Lehr, Monsey, N. Y.
Frank G. Lier, Columbia	Donald G. Huttleston, Brooklyn Botanic Garden
J. Herbert Taylor, Columbia	Hester M. Rusk, Brooklyn Botanic Garden

Newsletter on Microenvironment, Volume 1, No. 1, October 1954 has been issued by Dr. Robert B. Platt, of Emory University, Emory, Georgia, under direction from the Committee on Microenvironment of the Ecological Society of America. There are some 37 pages abstracting the research interest in this field of perhaps 150 scientists. The idea being that if you find someone doing work such as yours, you might care to exchange information, or beg some. Copies are 50 cents and information for No. 2 may be sent to the above editor.—J.A.S.

BULLETIN OF THE TORREY BOTANICAL CLUB
VOL. 82, No. 3, pp. 253–260 MAY, 1955

INDEX TO AMERICAN BOTANICAL LITERATURE

COMPILED BY
LAZELLA SCHWARTEN
WITH THE COLLABORATION OF THE EDITORS OF THE TAXONOMIC INDEX

TAXONOMY, PHYLOGENY AND FLORISTICS

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